PREPARATION AND QUALITY ANALYSIS OF YACON (*Smallanthus sonchifolius*) BASED HERBAL WINE

by

Samit Karki

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

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Preparation and Quality Analysis of Yacon *(Smallanthus sonchifolius)* Based Herbal Wine

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Samit Karki

Department of Food Technology

Central Campus of Technology, Dharan

Institute of Science and Technology

Tribhuvan University, Nepal

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Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This ***dissertation*** entitled ***Preparation and Quality Analysis of Yacon based Herbal Wine*** presented by **Samit Karki** has been accepted as the partial fulfillment of the requirement for the **B. Tech. degree in Food Technology**

Dissertation Committee

1. Head of the department _____

(Mr. Basanta Kumar Rai, Assoc. Prof.)

2. External Examiner _____

(Birendra Kumar Yadav, Asst. Prof.)

3. Supervisor _____

(Mr. Dev Raj Acharya, Lecturer.)

4. Internal Examiner _____

(Geeta Bhattarai, Assoc. Prof.)

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Samit Karki

Abstract

Wines were prepared by fermenting yacon juice, yacon pulp and yacon syrup musts maintained at 24°Bx TSS, 3.8 ± 0.1 pH and 100 ppm SO_2 for 20 days at 26 ± 2°C temperature. The wines were analyzed for their chemical and sensory characteristics and the best wine was selected. *Sarpaganda* (*R. serpentina*) and *Pakhanbed* (*B. ciliata*) root powders were separately added to the yacon juice must at the rate of 10 g/L, and effects of herbs addition on the chemical and sensory properties of yacon juice wine were studied.

Results showed that the total and fixed acidities were maximum in yacon syrup wine (0.86 and 0.72%, m/v as lactic acid) compared to yacon juice and pulp wines. No significant difference in TSS was found among yacoon wines. Antioxidant activity was highest in yaon syrup wine (89.83%), while it was lowest in yacon pulp wine (42.44%). Total phenolics content was more than 2-fold in yacon syrup wine (86.5 mg GAE/100 ml) compared to yacon juice and pulp wines. Yacon juice wine had significantly higher alcohol content (12.5%, v/v) than those of others. Total esters, total aldehydes and higher alcohol contents of the three wines ranged from 46.36 to 55.05 g ethyl acetate/100 L alc, 0.21 to 0.29 g acetaldehyde/100 L alc) and 17.0 to 22 mg/100 ml wine respectively. Methanol contents did differ among the wines (0.00155 ± 0.00015%, m/v). Sensory evaluation showed that wine made from yacon juice had relatively better sensory quality of all the wines. Chemical analysis showed that herbs addition had no significant effect on pH, TSS, fructose and volatile acidity of yacon juice wines. *Pakhanbed* added wine had significantly higher total phenolic content (103.5 mg GAE/100 ml) compared to control wine (42.7) and *Sarpaganda* added wine (47.1). Total reducing sugars (mg% as glucose) was lowest in yacon juice wine (115.7) compared to *Pakhanbed* (243.9) and *Sarpaganda* (251.4) added yacon juice wines. Addition of herbs had no significant effect ($p<0.05$) on alcohol, total esters, total aldehydes, fusel oil and methanol contents of yacon juice wine. Addition of *Pakhanbed* significantly enhanced the antioxidant activity of wine. Sensory evaluation indicated that *Pakhanbed* added yacon juice wine had higher sensory preference scores of all the wines evaluated. Therefore, addition of *Pakhanbed* at the rate of 5 g/L of yacon juice must could significantly enhance the medicinal value of wine without significantly affecting its chemical and sensory properties.

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List of abbreviations

Abbreviation	Full form
ADY	Active dry yeast
ATP	Adenosine triphosphate
CFU	Colony forning units
EMP	Embedem-Meyerhof glycolytic pathway
FOS	Fructooligosacharides
KMS	Potasium metabisulfite
LSD	Least significant difference
MLF	Malolactic fermentation
NADH	Nicotamide adenine dinucleotide
OIV	International organization for vine and wine
PPOs	Polyphenol oxidase
TSS	Total soluble solid

Part I

Introduction

1.1 General introduction

The term 'wine' is applied to the product made by alcoholic fermentation of grapes or grape juice, with an aging process. However, products of fermentation of others berries, fruits, and honey are also called wines and the resultant wine is normally named after the substrate used. Wine is one of the God's choicest gift to man and history is almost a romance. The oldest testament in the Bible gives evidence of wine existing but there is a definite evidence of its use in China and Egypt in 2000 and 3000 B.C. respectively (Andrew, 1980). The earliest recorded winemaking activities appear on the archaeological record in various places around the globe as far back as 6,000 years ago. Wine is associated with festivity, bacchanalian excess and verdant bounty in art and literature (Payne, 2016).

Different types of raw materials have been used for the preparation of wine, either for flavor or for enrichment of wine with chief chemical constituents (Gubhaju, 2006). Yacon (*Smallanthus sonchifolius*) is a perennial plant which forms sweet-tasting underground tuberous roots. The roots vary greatly in shape and size; commonly they are 15-20 cm long and 10 cm thick. They come in different colors, brown, pink, purple and cream. One plant can produce more than 10 kg of roots. Their crunchy texture very much resembles that of an apple (Ojansivu *et al.*, 2011). Different herb incorporated wines are also on practices throughout the world. Herbs are plants that are grown either as a food (usually as a condiment), or because they have some use in treating diseases (or making them better), or for spiritual reasons (for example, their smell). The word herb comes from the Latin word *herba*, meaning grass, green stalks, or blades. Botanists use the word to mean any plant with soft, succulent tissues.

Sarpagandha is the dried root of *Rauwolfia serpentina* Benth. Ex Kurz (Family Apocynaceae) occurring wild in eastern Nepal at 1200 m. and also cultivated. It is used as hypnotic, sedative, reduces blood presuure, remedy in painful affections of the bowel (Rajbhandary, 1995). *Pakhanbed* is a perennial rhizomatous creeping herbs on rocks ledges with stout. The part used is rootsock. The root is useful in piles, tumors, urinary discharges, heart diseases, diseases of bladder and lungs (Yadav, 2016).

Famous herbal wines include all types of vermouth (infused with wormwood and other herbs) and quinquina (which includes quinine along with fruit and herbs one of the most famous of these is called “Lillet”). Other familiar herbal wines are mulled wine and sangria (Payne, 2016). Ginger wine, is an alcoholic beverage made from a blend of ground ginger (*Zingiber officinale*) and raisins fermenting by the yeast, *Saccharomyces cerevisiae*. It is a popular beverage in Europe (Rai, 2009).

Wine serves as an excellent vehicle for extracting some of the beneficial components of plants because of the solvent properties of its alcohol paired with the acidity that typically characterizes wines of a particular terroir. Wine’s usual 12 to 14% alcohol content not only causes less of an impact on the body’s systems than distilled spirits, but wine also comes with other nutritive benefits and elements that help the body break it down and process it (Payne, 2016). Various kinds of herbs and spices play an important role in alcoholic beverage production. They are used as enhancer, preservative an antioxidant sources (Yuwa-Amornpitak *et al.*, 2012).

1.2 Statement of the problem

Yacon is an underutilized fruit crop but it has great potential to become a profitable product in small scale farming using organic cultivation. In Nepal, yacon is being commercially produced in various parts of the country but still they aren’t being efficiently utilized in the market. Yacon itself is an abundant source of fructooligosaccharide (FOS). FOS is a type of sugar that has a lower calorie value than other sugar types and its intake favors the growth of health-promoting bacteria while reducing pathogenic bacteria populations. So far, research work on the production of yacon wine has been carried out by using yacon juice only and works regarding the possibility of preparing wines from yacon pulp and yacon syrup are scarce. Moreover, studies related to the preparation and quality analysis of herbal wines in Nepalese context has not been carried out so far. Information on the antioxidant activity of these wines is also scanty. With this view, the present study was undertaken to investigate the possibility of preparing yacon-based herbal wine using *Bergenia ciliata* (*Pakhanbed*) and *Rauwolfia serpentina* (*Sarpagandha*).

1.3 Objectives

1.3.1 General objective

The general objective of this dissertation was to prepare yacon-based herbal (*Pakhanbed* and *Sarpagandha*) wine and to evaluate its quality attributes.

1.3.2 Specific objectives

The specific objectives of the study are as follows:

1. To prepare yacon wines from juice, syrup and pulp of yacon.
2. To carry out sensory and physicochemical analysis of different Yacon wines.
3. To prepare herbal wine by incorporating *Sarpagandha* and *Pakhanbed* to the pre-selected best yacon wine's must.
4. To assess chemical and organoleptic characteristics of the herbal wines.

1.4 Significance of the study

Despite huge health benefits of yacon (*Smallanthus sonchifolius*), its cultivation has declined over the last few years owing, mainly, to its marketing problem. As the juice yield of yacon is comparable to those of apple and pears, and the TSS similar to that of pear, the outcome of this study will help in developing yacon as a promising raw material for wine making. Commercial utilization of yacon for wine making will be beneficial not only for the wine industries in finding alternative raw material, but also to the grower for ensured marketing.

Rauwolfia serpentina L. Benth Kur z, commonly called *Sarpagandha*, is mainly known for its phytochemical reserpine, which was widely used as an antihypertensive drug and a powerful sedative; and hence it has important medicinal values. The *Rauwolfia* species is mainly known for its phytochemical reserpine, which was widely used as an antihypertensive drug. Its alkaloid called reserpine is a powerful sedative; and hence it has important medicinal values. The root extract of this plant is very useful in disorders of gastro intestinal tract viz. diarrhea, dysentery and cholera and colic. The alkaloids found in roots are employed for treatment of several diseases such as heart disorders and even cancers (Deshmukh *et al.*, 2012). *Rauwolfia serpentina* has long being used in India for the treatment of snakebites, hypertension, high blood pressure and mental illness (Deyl and De, 2011).

Bergenia ciliata, commonly called as *Pakhanbed* is useful in piles, tumors, urinary discharges, heart diseases, diseases of the bladder and lungs. It is also used as tonic in fever, diarrhea, cough and dysentery (GON, 2016). Wine serves as an excellent vehicle for extracting some of the beneficial components of plants because of the solvent properties of its alcohol paired with the acidity that typically characterizes wines of a particular terroir. Therefore, inclusion of these herbs during wine fermentation will extract many antimicrobial and health promoting active components from the herbs resulting in the production of herbal/medicinal wine of longer self-life compared to other customary wines. Therefore, this research will help explore the possibility of incorporating *Bergenia ciliata* (*Pakhanbed*) and *Rauwolfia serpentina* (*Sarpagandha*) in producing herbal wine, thus promoting commercial cultivation of these endangered Nepalese medicinal plants.

1.5 Research hypotheses

1. There is no significant difference in the chemical and sensory characteristics among yacon juice, pulp and syrup wines.
2. Addition of *Bergenia ciliata* (*Pakhanbed*) and. *Rauwolfia serpentina* (*Sarpagandha*) will have no significant effects on the chemical and sensory properties of yacon juice wine.

1.6 Limitations of the study

1. The fermentation was done in ambient condition because of the unavailability of temperature control instrument in laboratory.
2. The fermentation was carried out at same TSS, temperature and adjustment of pH by addition of same acid. Hence, optimization on TSS, temperature and acid used was not done.
3. Prepared yacon wine was not aged properly due time and technical constraints.
4. Only one yeast type was used.

Part II

Literature review

2.1 Historical background of alcoholic beverage

The production and consumption of alcoholic beverage is one of the man's oldest activities. At every part of world, different civilization had developed some types of alcoholic beverages (Varnam and Sutherland, 1994). The use of wheat, rye, millet, rice, oats, barley, potatoes or grapes in early fermentation processes paved the way to the technologies that are in existence currently (Jones, 1995). Wine making was an important economic activity in the countries of the 'old world' such as France and Germany (Varnam and Sutherland, 1994). Today, brewing, wine making and distilling are of major commercial importance in many non-islamic countries and, through taxation, these activities can be an important Source of government revenue (Reed, 2004).

Alcoholic fermentation was first identified by Gay Lussac in 1810, but at that time yeast was not recognized as causative organism. Schwan in 1835 demonstrated that yeast could produce alcohol and carbon dioxide when introduced in sugar-containing solution. He termed yeast *Zuckerpilz* meaning sugar-fungus, from which the name *Saccharomyces* originated. *Saccharomyces* group possesses almost all the credit of producing alcoholic beverages (Reed, 2004).

The yeast cells growing under anaerobic conditions caused the conversion of glucose to alcohol and researchers also demonstrated that fermentation could be carried out using cell free juice, which led to the discovery of the role of enzymes in fermentation. He called the enzyme zymase. Such work of pioneers finally revealed the truth that the alcoholic fermentation was in fact anaerobic, due to the presence of an enzyme complex known as zymase, which is made available by the yeasts. Having realized the importance of yeasts in fermentation, people started culturing valuable yeasts and exploiting them for the production of various alcoholic beverages. Today, yeasts are utilized throughout the world for the production of alcoholic beverages in many different forms and tastes. The starting materials normally comprise either sugary materials, which need to be hydrolyzed to simple sugars before fermentation (Buglass *et al.*, 2011).

Over the year, a vast range of alcoholic beverages have evolved although, in most cases, it is possible to place these in one of three categories- beer, wine or distilled spirit – according to ingredient and method of manufacture (Varnam and Sutherland, 1994).

In Nepal, the history of alcoholic beverage dates back to ancient times. These technologies were developed by ethnic groups while celebrating various festivals and settlement of marriage. The knowledge of home brewing has been passed on to generations but they are quite ignorant about the broad dimensions of microbial biochemistry or their complex mechanisms. In fact the exact nature of fermentation is still not fully known to them (Gubhaju, 2006).

2.2 History of wine making

The history of wine and winemaking is as old as civilization itself. Viticulture, or grape-growing, began in Georgia some 9000 years ago from here it spread to Middle East via the Tigris and Euphrates rivers to Mesopotamia, and then on to Persia. Stories abound about how wine was first discovered, and one of the more delightful tells of a mythical Persian king called Jamsheed. At his court, grapes were kept in jars for eating out of season. One jar was discarded because the juice had lost its sweetness and the grapes were deemed to be poisonous. A damsel from the king's hareem was suffering from nervous headaches and tried to take her life with the so-called poison. She fell asleep and awake later feeling revived and refreshed. She told everyone what she had done and of the miraculous cure, and thereupon a quantity of wine was made and his court drank of the new beverage. And that is it in a nutshell. Someone, somewhere in Asia Minor, possibly in modern Anatolia or Georgia, put wild grapes in a container, which were pressed by their own weight. The resulting juice began to ferment and a new drink was discovered that was to give untold pleasure to an untold number of people. Also the great civilizations of ancient Greece, and Rome trace wine back into their pre-history, with similar legends about its discovery (Sandler and Pinder, 2003).

2.3 Wine industry and market in Nepal

Wine is considered to be the third most consumed liquid after water and beer (Aasha *et al.*, 2018). In Nepal, the history of commercial wine making is not very long (Bhandari, 1992). Although the practice of making some forms of traditional wines can be traced to times immemorial. There is drastic change in wine drinking culture in Nepal within few years (Khatiwada, 2015). The increasing number of wine consumers in Nepal shows the string influence of western culture in Nepal. While Nepal had been brewing local home brewed alcoholics since the beginning of ethnic civilizations, it wasn't long before that sophisticated drinks like beer, whisky and vodka manufacture were.

In Nepal, many young generations are drinking wine over beer or other beverages. It is estimated that there is about 150% increase in wine consumption since 2007 and it goes on increasing. Wine drinking has become as a fashion in Nepal. Most of the Nepalese consumer doesn't know the social and health benefits of drinking wine, Nepalese consumers just drink to show they are adopting western culture, modernizations and to show their higher status in the society. As we know wine itself is a 'culture', has social, cultural and health benefits if taken correctly. In many religions, wine was used and still being used as an offering to the god. It was estimated that over 450000 liters of wine is consumed in Nepal in 2009 (Aasha *et al.*, 2018)

More than 50 brands of wine are produced in the country. Brands like Hinwa, Dandaghare and Divine hold a major share of the market, while recently launched Black Stone and Moon Dance are struggling to gain fans within the short period of time and the consumers of Nepali wines have grown significantly. No one used to take a glance at the Nepal made wine bottle five years ago, while around 100,000 bottles of Nepal wines are on demand in the market (monthly) (Nepal, 2014).

Following are some of the popular brands of wines made in Nepal (Rijal, 2016).

I. **Dadaghare**

The wine manufactured in Pokhara. Dadaghare, is considered to be the first Nepali wine. It is not only popular among the local customers but also foreigners. The wine is available in four different flavors- *Aangan*, *Pidi*, *Majheri* and *Aati*, that are manufactured using various fruits, herbal fruits and honey and are absolutely chemical free.

II. **Hinwa**

One of the most popular wines, Hinwa, is manufactured by Makalu wine industries at Sankhuwasabha. It is made from wild fruits like raspberry, Himalayan barberry and saffron. This industry first started manufacturing wines in 1995.

III. **Netlange**

Manufactured by Sakaro Beverages, Nettlange, is one of the popular Nepali wines in the local market. It is made from nettles (*Sishno*) and oranges.

IV. **Grapple**

Made from black grapes that are imported from India, and apples from Mustang, Grapple is manufactured by Sakaro Beverages.

V. **Divine**

Divine wine is one of the fast selling brands available in the market and it was introduced in 2010. The wine is manufactured by Shree Mahakali wine industry, is made of grapes, spices, tea and various other fruits.

2.4 Classification of wine

Classifications of wines based on different characteristics are shown in Table 2.1.

Table 2.1 Classification of wine

Basis of classification	Class/type	Description	Example
Color	Red wine	Contain the red coloring matter of skin, pulp and seeds	Burgundy
	White wine	Do not contain the red coloring matter of skin, pulp and seeds	Rhine wine
	Pink wine	Low concentration of red coloring matter is maintained	Rose
Relative sweetness	Sweet wine	Contain up to 7% sugar	Sherry (sweet)
	Dry wine	Contains less than 0.12% sugar	Sherry (dry)
Alcohol content	Natural	Contains 8.5 – 16% alcohol by volume (% abv)	Table wines
	Fortified	Contains 17 – 21% abv	Sherry
Effervescence	Still	Does not contain CO_2	Chianti
	Sparkling	Contains CO_2 (natural or added)	Champagne
Wine Advisory Board, USA	Dessert wine	Contains sugar; taken after meal	Sherry (sweet)
	Appetizer wine	Dry; fortified; taken before meal	Sherry (dry)
	Sparkling wine	Contain CO_2	Champagne
	Red table wine	Natural; red in color	Chianti
	White table wine	Natural; pale yellow to straw color	Rhine wine

Note: There is considerable overlapping of wine types in the classification shown above.

For example, a Red Table wine can at the same time is sweet, sparkling, fortified, or natural. Similarly, a fortified wine can be sweet, sparkling, red, or white (Rai, 2012).

2.5 Chemical composition of wine

2.5.1 Alcohol

2.5.1.1 Ethanol

There are many different kind of alcohol, but when the term is used loosely by winemakers, it is invariably applies to the potable alcohol called ethyl alcohol or ethanol, the common ingredients of alcoholic drinks of all type. Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Simple sugars are the raw material. Zymase, an enzyme from yeast, changes the simple sugars into ethanol and carbon dioxide. The ethanol produced by fermentation ranges in concentration from a few percent up to about 14%. Above about 14%, ethanol destroys the zymase enzyme and fermentation stops. Ethanol melts at –114.1℃, boils at 78.5℃, and has a density of 0.789 g/ml at 20°C. It mixes easily with water in any proportion, and where quantities are mixed there is a contraction in volume. It is clear, colorless, inflammable liquid. It is good solvent for essential oil, ester, tannins, various organic acids and certain other organic compounds. It burns easily in air, so that oxidation is possible and then gives a blue smokeless flame, producing water and CO_2 (Shakhashiri, 2009).

A range of alcohols is present in wine. The most important of these is ethanol. Although small quantities are produced in grape cells during carbonic maceration, the primary source of ethanol in wine is yeast fermentation. Ethanol is crucial to the stability, aging, and sensory properties of wine. The inhibitory action of ethanol, combined with the acidity of the wine, permits wine to remain stable for years in the absence of air. Ethanol has multiple effects on taste and mouth-feel. It adds directly to the perception of sweetness. It indirectly modifies the perception of acidity, making acidic wines appear less sour and more balanced. At high concentrations, alcohol produces a burning sensation, and may contribute to the feeling of weight (body), especially in dry wines. Ethanol can also increase the intensity of bitterness, decrease the astringency of tannins and influence the volatility of aromatic compounds. In addition to helping to dissolve pigment and tannin extraction from grapes, it is a solvent for

many volatile compounds produced during fermentation, and formed during maturation in oak cooperage (Jackson, 2014).

2.5.1.2 Methanol

Methanol occurs in wine, but only in trace amounts. Within its normal range (0.1–0.2 g/L), methanol has no sensory or health consequences. Of the over 160 esters found in wine, few are associated with methanol. Health concerns connected to methanol relate to its metabolism to formaldehyde and formic acid. Both are toxic to the central nervous system. One of the first targets of formaldehyde toxicity is the optic nerve, causing blindness. However, methanol never accumulates to toxic levels in wine, at least under legitimate winemaking procedures. The marginal amount of methanol that is found in wine comes almost exclusively from the demethylation of pectin. These methyl groups are released as methanol. Thus, methanol content is a partial function of the must pectin content. Unlike most fruits, grapes are low in pectin content. Thus, wine has the lowest methanol content of any fruit-based, fermented beverage. However, pectolytic enzymes, added to juice or wine as a clarification aid, can inadvertently increase the methanol content. Adding distilled spirits to a wine may also slightly increase the methanol content (Jackson, 2014).

2.5.1.3 Higher alcohols

Alcohols with more than two carbon atoms are commonly called higher alcohols or fusel oil. They commonly account for about 50% of the aromatic constituents of wine, excluding ethanol. The principal higher alcohols produced by yeast are the aliphatic alcohols n-propanol, isobutanol (2-methyl-1-propanol), active amyl alcohol (2-methyl-1-butanol), isoamyl alcohol (3-mthyl-1-butanol), and the aromatic alcohols hexanol and 2- phenethyl alcohol. The higher alcohols content in wine should be 80-540 mg/L and the concentration of higher alcohols below 300 mg/L contributes the desirable aroma of wine, whereas these components are seen as a negative factor in creating the aroma when their level exceeds 400 mg/L (Usansa, 2003).

The higher alcohols are important as the immediate precursors of more flavor active esters, so that the control of higher alcohol formation needs regulation to ensure that the ester production is controlled. The higher alcohols are produced by yeast as secondary metabolites of amino acid metabolism. The situation is actually complicated by the fact that yeast cells are capable of synthesizing their own higher alcohols from other pathways rather than from

amino acids. Again, as for esters, yeast strain turns out to be the most important factor. Conditions which favor increased yeast growth, such as excessive aeration or oxygenation, promote higher alcohol formation, but this can be ameliorated by the application of a top pressure during fermentation (Baxter and Hughes, 2001).

2.5.2 Esters

There are a number of esters which contribute to the flavor of wines. Ester plays an important role in the formation of wine's sensory characteristics. They are formed from acids and alcohols during wine fermentation and ageing process. There are a lot of different alcohols and acids in wines, so the number of possible ester is also very large. Ester in wine have two distinct origins; enzymatic esterification during the fermentation process and chemical esterification during long term aging (Usansa, 2003). Biosynthesis of esters mainly depends on fruit maturity, yeast species, must aeration, fermentation technology and temperature. Their amount in young wines varies over a wide range (from 25 to 300 mg/L). The majority of esters are formed at the beginning of fermentation, and during wine maturation their concentration changes only slightly. Among wine esters very important in terms of bouquet are isoamyl acetate (banana aroma), 2-phenylethyl acetate (rose aroma), and ethyl acetate (strong, sweet aroma) (Clarke and Bakker, 2004).

2.5.3 Aldehydes

Acetaldehyde is of special interest because of its role as the immediate precursor of ethanol. It has an unpleasant 'grassy' flavor and aroma. Acetaldehyde is formed during the early to mid-stages of fermentation and thereafter it declines to a low level. In some circumstances, it can accumulate during fermentation in concentrations above the flavor threshold of 10-20 ppm. The principal causes of high acetaldehyde concentrations in wine are the use of poor quality pitching yeast, excessive must oxygenation, unduly high fermentation temperature and excessive pitching rates (Briggs *et al.*, 2004).

Generally, white and red wines have similar aldehyde contents. The aldehyde content is however, low and this may be explained by the fact that the sulphur dioxide added to wine reacts with aldehydes to form a-hydroxysulphonic acids, which reduce the free aldehyde content. Furthermore, aldehydes can be chemically bound to ethanol and higher alcohols as acetals. White and red wines produced in various countries contain 1-propanol (11-125 mg/L), 2-methyl-1 propanol (15-174 mg/L), 2-methyl-1-butanol (12-311 mg/L) and 3-

methyl-1-butanol (isopentanol; 49-180 mg/L). Aldehydes also play a role in color, by reacting with sulfites and preventing bleaching, and more importantly, by participating in the binding of anthocyanins to tannins and stabilizing color. Finally, aldehydes also play a role in texture, due to the above participation in tannin polymerization reactions (Frivik and Ebeler, 2003). Chemical composition of some commercial wines is given in Table 2.2.

Table 2.2 Chemical composition of some wines

Parameters	Port	Sherry	Claret	Burgundy	Champagne
Specific gravity	0.995-1.050	0.992-1.015	0.995-1.001	0.995-1.001	1.040-1.055
Alcohol (g/100 ml)	13.5- 20.0	13.5- 20.5	7.5- 12.5	7.5- 12.5	10.0- 14.0
% Total solid	3.3-13.0	20.-9.6	2.0-3.5	2.0-3.5	9.5-18.0
% Free volatile acid (as acetic acid)	0.05-0.10	0.15-0.23	0.09-0.15	0.2-0.35	0.03-0.20
% Fixed acid (as acetic acid)	0.35-0.55	0.25-0.50	0.30-0.50	0.3-0.60	0.30-0.45
% Ash	0.25-0.35	0.35-0.55	0.20-0.30	0.2-0.4	0.25-0.45
% Sugar (as sucrose)	2.5-12.0	2.0-7.0	0.0-0.7	0.03-0.55	8.5-16

Source: Egan *et al.* (1981)

2.6 Some research works conducted in wine

Ancin *et al.* (1996) studied the influence of pre-fermentation clarification on the higher alcohol contents of wines. He reported that the pH, reducing sugars (g/L), total acidity (g/L as tartaric), and volatile acidity (g/L as acetic) contents in rose and white wines were 3.10, 0.99, 4.95 and 0.3; and 3.33, 1.67, 3.89 and 0.27 respectively. Similarly, the ethyl alcohol (%, v/v) and higher alcohol (mg/L) contents in rose and white wines were 12.5 and 203; and 10.6, and 257 respectively.

In addition, grape variety and ripeness affect the concentration of higher alcohols (Cabrera *et al.*, 1988), probably because of the existence of qualitative and quantitative differences in must amino acid composition (Ough and Bell, 1980; Vos, 1981; Herraiz *et al.*, 1989, Rapp and Versini, 1991). Also, the presence of large amounts of insoluble solids in musts during fermentation produces wines with high levels of higher alcohols and esters when compared to wines made with clarified musts (Groat and Ough, 1978).

Vilanova *et al.* (2007) studied on aromatic compounds in wines produced during fermentation: effect of three red cultivars. They reported that the ethanol (%, v/v), total acidity (g/L as tartaric acid), volatile acidity (g/L as acetic acid), reducing sugar (g/L) and total phenolics (g/L as gallic acid) in Caino Longo, Caino Tinto and Caino Bravo wines were 9.46, 9.30, 0.30, 0.70 and 35.81; 9.16, 9.10, 0.30, 0.70 and 36.19; and 7.86, 10.30, 0.30, 1.00, 55.66 respectively. Similarly, the methanol (mg/L) and ethyl acetate (mg/L) contents in Caino Longo, Caino Tinto and Caino Bravo wines were 88.69 and 28.51; 135.62 and 22.88; and 214.87 and 58.90 respectively.

Veeranjaneya Reddy *et al*. (2008) studied on wine production by novel yeast biocatalyst prepared by immobilization on watermelon (*Citrullus vulgaris***)** rind pieces and characterization of volatile compounds. He reported that the ethanol content was 4 g/L and the concentrations of ethyl acetate and methanol were not more than 100 mg/L in all cases.

Lee and Cooley (1981) studied higher alcohol contents in New York wines and reported that the higher alcohol contents in red and white wines were 339 and 188 mg/L respectively.

Reddy and Reddy (2009) studied effects of enzymatic maceration on synthesis of higher alcohols during mango wine fermentation and the following results were reported which is given in Table 2.3.

Table 2.3 Effects of enzymatic maceration on synthesis of higher alcohols during mango wine fermentation

Content	Banginapalli		Totapari	
	Untreated	Treated	Untreated	Treated
Ethanol (%,w/v)	6.3	8.5	5.1	7
Higher alcohol (Fusel oil, mg/L)	265	340	273	358
Total etsers (ethyl acetate, mg/L)	20	32	16	25
Residual sugar (g/L, as glucose)	10	2.5	15	3
Acidity (%, w/v)	0.45	0.66	0.36	0.54
pH	4.4	4.7	4.5	4.2

Source: Reddy and Reddy (2009)

Alvarenga *et al.* (2011) studied potential application of *Saccharomyces cerevisiae* strains for the fermentation of banana pulp and following results were reported which is given in Table 2.4.

Table 2.4 Potential application of *Saccharomyces cerevisiae* strains for the fermentation of banana pulp

Strain	Total reducing sugar (g/L)	Ethanol (%, v/v)	Final acidity (g acetic acid/100 ml)	pH
Commercial yeast	3.125	7.84	0.49	4.30
UNICAMPV1	1.20	6.47	0.61	4.34
UFMGA905	4.82	5.64	0.51	4.35
UFMGA1007	4.10	5.68	0.56	4.29
UFMGA1240	6.13	5.34	0.46	4.40

Source: Alvarenga *et al.* (2011)

Oliveira *et al.* (2011) studied fruit wine produced from cagaita (*Eugenia dysenterica* DC) by both free and immobilized yeast cell fermentation. He reported that the ethanol content (g/L), higher alcohols (µg/L) and ethyl ester concentrations in free and immobilized cells fermentation were 94.63, 86.82, 82.086 and 1511.42; and 94.94, 87.21, 37 and 812.17 respectively.

2.7 Wine yeast

Wine yeast is the member of the *Saccharomyces cerevisiae* group. This originates from the Greek words *Sakchar means* sugar and *mykes* fungus, referring to the strong sugar fermenting properties of the genus in general. Although, Hansen regarded them as a separate species, they are more ellipsoidal in shape than the round or ovate cells of brewery and bakery yeasts. Hansen restricted the name *S. ellipsideus* to them. In the nomenclature of Dutch school, these

yeast are classified as a variety of *Saccharomyces cerevisiae* and consequently named *S. cerevisiae* var. *ellipsideus*. In general articles, however, one will see them briefly described a ellipsoidal yeasts or true wine yeasts (Raut, 2014).

Wines can be prepared using either natural yeast flora of the grapes (spontaneous fermentation) or pure cultures (culture yeasts). Many manufacturers still depend on spontaneous fermentation which can produce wine of unique quality in terms of bouquet because the end product is the result of interaction of diverse yeast types. Each yeast type will contribute unique flavor to the wine. But yeast profile is diverse and spontaneous fermentation may sometimes lead to failure. Most strain of yeast do not produce large amount of wine as well few strains produce undesirable organic compounds such as organic acids, H_2S, higher alcohols, etc., that may affect the flavor (Rai, 2012). Nowadays the must is partially 'sterilized' by the use of sulphur dioxide, a bisulphate or a metabisulphite, which eliminates most microorganisms in the must leaving wine yeasts. Yeasts are then inoculated into the must. The yeast which is used is *Saccaromyces cerevisiae* var, e*llipsoideus* (synonyms: *S. cerevisiae, S. ellipsoideus, S. vini*.) Other yeasts which have been used for special wines are *S. fermentati*, *S. oyiformis* and *S. bayanus* (Okafor, 2007). Good wine yeast is one which will impart a vinous or fruit like flavor, will ferment sugar to a low content producing 14-18% alcohol, and is characterized by remaining in suspension during fermentation and then agglomerating to yield a coarse granular sediment that settles quickly and is not easily disturbed in racking (Pederson, 1980). In general, good wine yeast should have the following properties (Okafor, 2007):

a) High alcohol tolerance, i.e. the yeast should continue to ferment despite the increasing concentration of the alcohol, giving stronger, drier wines with up to 16% alcohol (v/v), or even up to 18% (v/v) where the yeast is fed by periodic additions of sugar in small amounts.
b) Good degree of agglutination i.e. the tendency of the yeast to flocculate into small lumps that give a cohesive sediment as fermentation ceases, so that racking is simple and the wine clears easily.
c) Steady, persistent fermentation capacity; this leads to wines of better quality than when the fermentation falls away after a tempestuous start.
d) Absence of unpleasant flavors generated by dead and dying cells.

e) Growth at the relatively high acidity i.e., low pH of grape juice or must for fermentation.
f) Osmotolerance, i.e. yeast should able to tolerant high osmotic pressure created by high concentration of sugar on must composition.
g) SO_2 tolerance, i.e. for partial sterilization of must SO_2 in the form of sulfite is used yeast should not be affected by applied sulfite.

There are two reasons for using starters. One is to start the alcoholic fermentation quickly after the harvest. Indeed, in some cases, and preferably at the beginning of the winemaking the yeast population is too low (less than 10^4 CFU/ml). Multiplication up to 10^6 and more takes several days especially if the temperature is low. During this time, other microorganisms can develop, yeasts with oxidative metabolism and acetic acid bacteria that take advantage of the presence of oxygen to produce volatile acidity and many other defects. Thus, inoculation with starters at the concentration of 10^6 CFU/ml prevents the growth of such microorganisms. The second reason for the winemaker to use yeast starters is to improve the final phase of alcoholic fermentation. Indeed, grape musts are so rich in sugar and sometimes so poor in essential nutrients that yeast cannot survive long enough to ferment all sugars. Stuck fermentation is one of the major problems in winemaking. Hence the use of selected yeast starters allow a better control of the process as well influence on the sensorial and hygienic quality of wine (Lonvaud, 2002).

2.8 Alcoholic fermentation

Alcoholic fermentation is the anaerobic transformation of sugars, mainly glucose and fructose, into ethanol and carbon dioxide in presence of nitrogen compound. Fruit juices have the highest sugar concentration among the many substrates used for the production of ethanol by fermentation. As a result, the level of ethanol is among the highest seen and the importance of substrate and ethanol inhibition This process, which is carried out by yeast and also by some bacteria can be summarized by this overall reaction:

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2\,C_2H_5OH + 2CO_2$$

Hexose — Ethanol — Carbon dioxide

However, alcoholic fermentation is fortunately a much more complex process. At the same time as this overall reaction proceeds, a lot of other biochemical, chemical and

physicochemical processes take place, making it possible to turn the grape juice into wine. Besides ethanol, several other compounds are produced throughout alcoholic fermentation such as higher alcohols, esters, glycerol, succinic acid, diacetyl, acetoin and 2, 3-butanediol. Simultaneously, some compounds of grape juice are also transformed by yeast metabolism. Without the production of these other substances, wine would have little organoleptic interest (Zamora, 2009).

2.8.1 Biochemistry of alcohol fermentation by yeast

In wine, *Saccharomyces* metabolize glucose and fructose to pyruvate via the glycolytic pathway. One molecule of glucose or fructose yields two molecules each of ethanol and carbon dioxide. The particular enzyme present in the yeast has the general name zymase, but, in fact, yeast contains several enzymes, including invertase, which is necessary to split the sucrose into its component sugars (glucose and fructose). The mechanism of the metabolic pathway from glucose and fructose to ethyl alcohol has been well established; the conversion proceeds primarily via the Embden–Meyerhof glycolytic pathway oxidation to pyruvate, then to acetaldehyde and ethyl alcohol. For growth and reproduction, yeast cells require a steady supply of ATP (adenosine triphosphate) together with the reducing power of NADH (nicotinamide adenine dinucleotide). There are metabolic intermediates, which result in the noted formation of succinates, glycerol, acetoin, diacetyl, acetic and succinic acids. Notably, the production of alcohol during fermentation assists the physical extraction of numerous compounds (e.g. Terpenes) from grape cells, which appear in the fermented wine (Clarke and Bakker, 2004).

The organism uses EMP pathway, generating 2 ATP per mole of glucose converted to ethanol, plus CO_2. Ethanol, which is the end product, is primary metabolite. In an industrial fermentation, the basic strategy is to maintain Crabtree effect during the fermentation. A truncated form of the metabolic pathway for ethanol synthesis is given in Fig. 2.1.

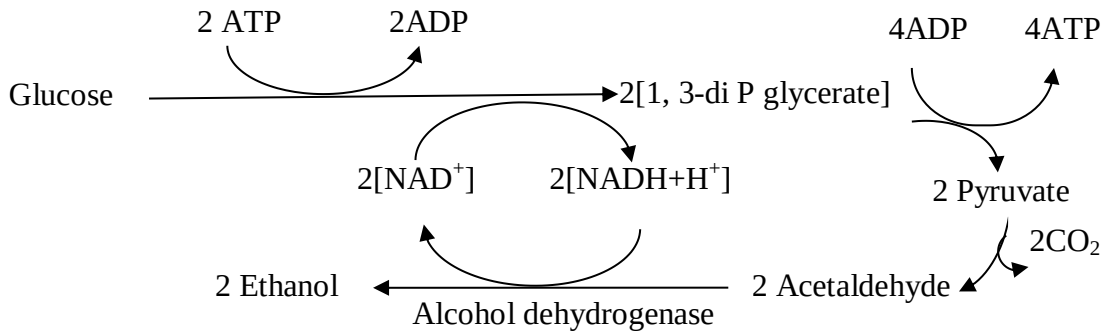

Fig. 2.1 Simplified pathway of alcohol synthesis by yeast

Source: Rai (2012)

2.8.2 Stoichiometry

Ethyl alcohol is the product obtained from alcoholic fermentation of the sugar by the action of enzyme *zymase* in the yeast. In alcoholic fermentation one molecule of glucose produce two molecules of ethyl alcohol and carbon dioxide.

2.8.3 Malo-lactic fermentation

Malolactic fermentation (MLF) in wine is by definition the enzymatic conversion of malic acid to lactic acid, a secondary process which usually follows primary (alcoholic) fermentation of wine but may also occur concurrently. But, this reduction of malic acid to lactic acid is not a true fermentation (Costantini *et al.*, 2009). The MLF occurs as a result of metabolic activity by certain lactic acid bacteria and results in the conversion of malic acid to lactic acid. The bacteria may also impact the flavor and aroma of the wine. Although spontaneous MLF may occur due to bacteria naturally present in musts and wines, specific starter cultures of bacteria are now commonly used as they allow more control over the process with more reliable results (Osborne, 2010). MLF is mainly performed by *Oenococcus oeni*, a species that can withstand the low pH (<3.5), high ethanol (>10 vol %) and high SO_2 levels (50 mg/L) found in wine. More resistant strains of *Lactobacillus*, *Leuconostoc* and *Pediococcus* can also grow in wine and contribute to MLF; especially if the wine pH exceeds 3.5. Wines with low levels of acidity should be protected from malo-lactic fermentation: wine

quality decreases if the acid level falls too low as well uncontrolled MLF also presents a risk of wine spoilage by compounds that can produce off-flavors (including acetic acid, volatile phenols and mousiness) or that may be hazardous to human health (Costantini *et al.*, 2009).

Malo-lactic fermentation can be easily prevented by early racking, cold storage, and maintaining 100 ppm or more of SO_2. On the other hand, if such fermentation is desired it can be facilitated by leaving the wine on the *lees* (yeast sediments) for prolonged periods at higher temperatures. This storage causes lysis of yeast cells and releases amino acids and other nutrients needed for the growth of the 'contaminant' lactic acid bacteria. This fermentation is particularly useful if the titrable acidity of the wine is to be reduced malo-lactic fermentation has an important bearing in the quality of wine. It is a natural way of reducing acidity in wine (Rai, 2009). The biochemistry of fermentation is given in Fig. 2.2

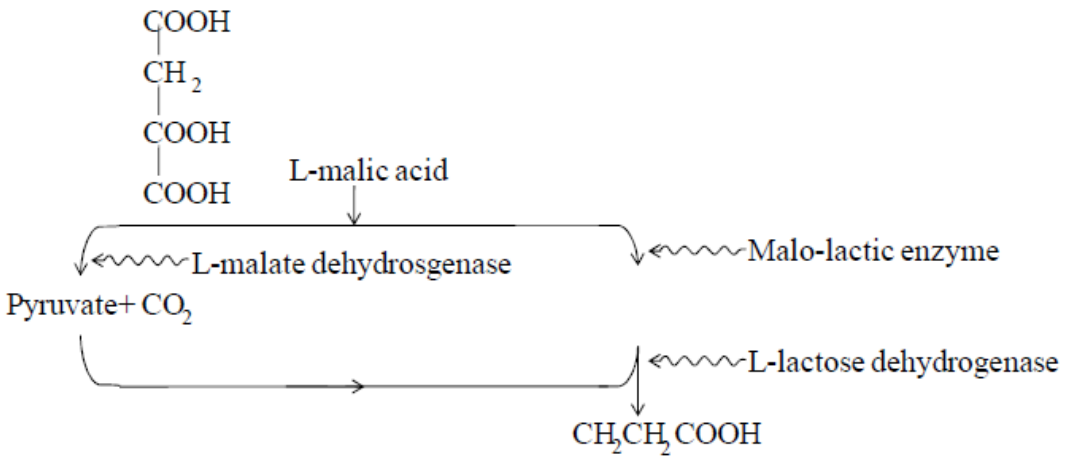

Fig. 2.2 The malolactic pathway

Source: Rai (2012)

2.9 General cultural condition for alcoholic fermentation

Cultural condition refers to the environment of yeast i.e. Fermentative media on which the propagation of yeast as well as final quality of wine is largely depended (Reed, 2004). Following are the few parameters, which determine cultural condition of the fermentative media.

2.9.1 pH

The pH of wine is crucial not only to its flavor but also to nearly every aspect of the wine. The pH could affect flavor, aroma, color, tartrate precipitation, carbon dioxide absorption, malolactic fermentation, stability, agility, and fermentation rate. Also, the pH can influence many chemical reactions that take place in wine. The optimum pH for wine production varies from types of fruits and type of wine that should be made, pH range of 2.8 to 4 cover most wines (Butzke, 2010). At higher pH the concentration of glycerin is increased during fermentation and at lower pH there is a noticeable effect of log phase (Reed, 2004). The pH higher than 4.0 are generally avoided as spoilage is more likely to occur above this level. Many wine makers keep wine pH below 3.65 (Rotter, 2008). A low pH increases the efficacy of many preservatives such as sulfur dioxide and sorbic acid. The most common adjustment to must pH is to lower it through the addition of acids like malic, citric, and tartaric acid. Tartaric acid is the most recommended acid for must adjustments because, it is a stronger acid than malic and citric acid and less susceptible to breakdown by microorganisms during the alcoholic and malolactic fermentations as well (Butzke, 2010) Wine is a highly buffer liquid. This means that the corresponding pH decrease for a given addition in titrable acid (added acidity) is not directly proportional. Further, the change in pH for a given titrable acidity increase/decrease is unique to each individual wine, since every wine is buffered slightly differently. However, as a general rule, the addition of 0.5-1 g/L acid as tartaric tends to drop the pH by about 0.1 units (Rotter, 2008).

2.9.2 Temperature

Temperature plays important role on fermentation. Above 38°C the yeast will certainly be killed; at too low a temperature it will ferment only very slowly. In general temperature of primary fermentation should be 20°C, temperature of secondary fermentation should be 15°C and finish wine storage temperature should be 10°C (Berry, 1996). The optimum temperature for the fermentation is dependent upon the types of wines produced. For white wine the temperature is 10-15°C and that for the red wine is 20-30°C. There is possibility of stuck fermentation if it is carried at higher temperature. On the other hand, low temperature may delay onset of fermentation. At high temperature, the loss of alcohol and aroma substance takes place. Also, a large amount of by product like glycerol, acetaldehyde may be formed. An imbalance of these constituents can be very detrimental to wine quality. It has been reported that at higher temperature the formation of higher alcohol decreases. The advantage

of lower fermentation temperature are the fresher and fruitier character of wine, smaller losses of ethanol and less danger of producing volatile acidity (Reed, 2004).

2.9.3 Sugar concentration

The 'must' having very high sugar concentration imparts high osmotic pressure, which in turn has a negative effect on yeast cells, since both growth of yeast and fermentation activity are lowered. The optimum sugar concentration in terms of total soluble solid is 20-24°Bx. The tolerance of higher sugar concentration varies according to the yeast species (Reed, 2004).

2.10 General method of wine preparation

Wild yeast and other microorganisms are present on the skin of the grapes and these pass into the juicy pulp (known as must) when the fruit is crushed. These are destroyed by adding sulphur dioxide (or KMS) in the required quantity. If the sugar content is low, sucrose is added to the desired strength and the pH is adjusted to 2.8 to 4 by the addition of tartaric acid. Next, the must is inoculated with a pure culture of actively growing yeast (*S. ellipsoideus*). The temperature and duration of fermentation depend upon whether dry or sweet wine is required. Fermentation usually lasts 4 to 10 days. When fermentation is complete, the clear wine is siphoned from the yeast sediment into barrels (racking) and the wine allowed to age. During this period, secondary fermentation takes place and wine also losses it's raw and harsh flavor and mellows down. During this period of maturation, clarification takes place in natural way. It can also be achieved by fining and filtration. Next, the wine is bottled and allowed to mature; the time of this maturation extends to a number of years depending upon the quality desired (Mmegwa, 1987). A simplified flow-sheet of wine preparation is given in Fig. 2.3.

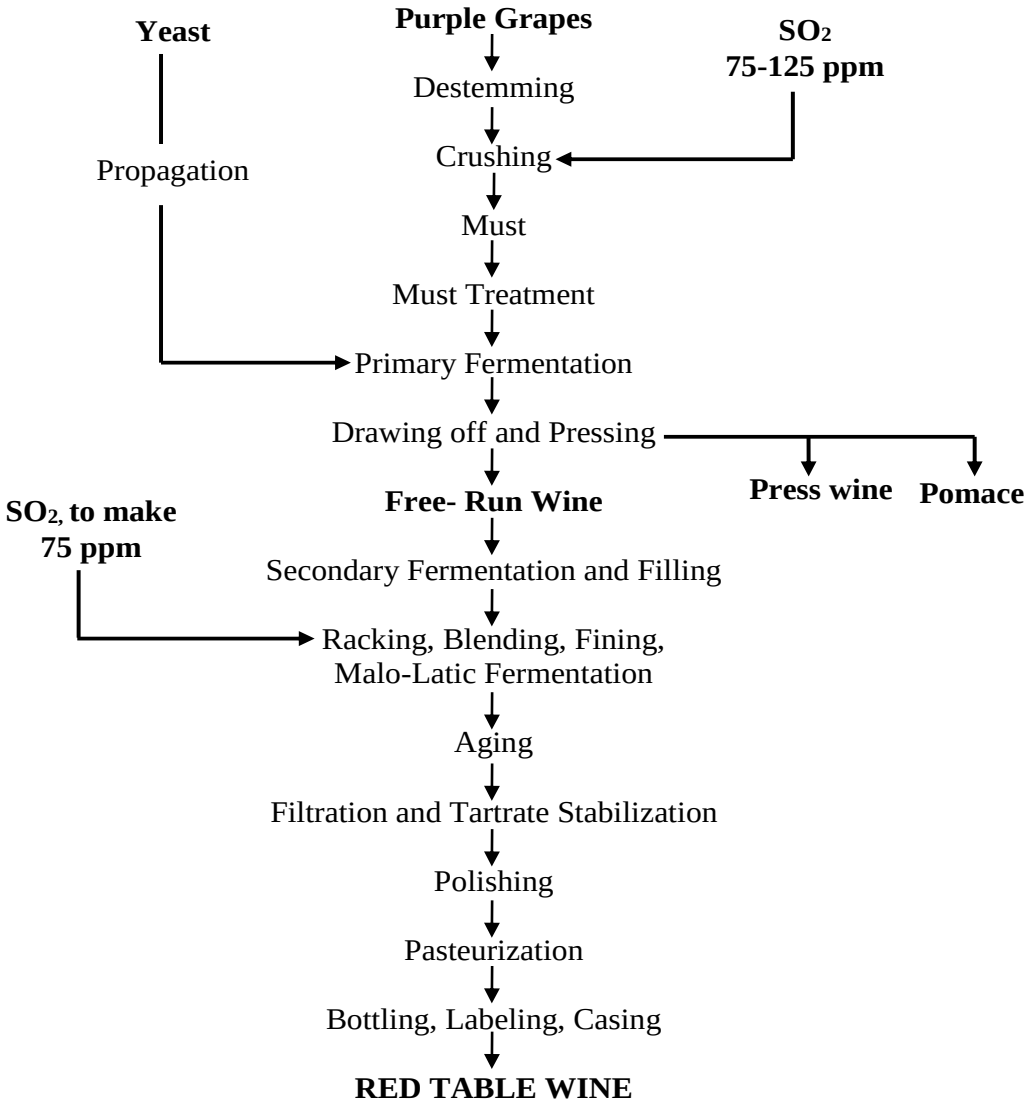

Fig. 2.3 Flow chart of red table wine preparation

Source: Rai (2012)

2.10.1 Selection of raw material

Any suitable raw material is chosen to function as a substrate. Compared to cereals, fruit juices are more readily utilizable substrate by yeasts for the alcoholic fermentation. The latter is also a suitable media for the yeast to grow (Varnam and Sutherland, 1994). Following criteria should be fulfilled when selecting for proper raw material for fermentation (Reed, 2004).

- It should be readily available.
- It should be good source of carbon and nitrogen.
- It should have sufficient amount of fermentable sugar.
- It should not contain any toxic compound nor should impart any undesirable odor or taste.
- It should be clean, sound and mature.

2.10.2 Crushing and blending

This step is carried out to extract the juice from the fruit (Okafor, 2007). Three types of crusher are generally used: Roller type, disintegrator type, and garolla type the last one is more generally used (Rai, 2012). It has been suggested that the process should be very gentle. If the blending and crushing machine is constructed of mild steel or cast iron then iron causes "ferric cause-cloudiness" of wine due to iron; actually iron will react with the tannin of the juice to form ferric-tannin complex. Bronze equipment is also used but may cause dissolution of copper and tin from bronze equipment and it will affect the color. Usually, stainless steel is used for the crushing machine. Water may be added during blending/crushing for smoothness of operation (Reed, 2004).

If the must does not meet the requirement, grape juice concentrate, sugar, acid, etc., most is added for the adjustment. This manipulation to standardize the must is called amelioration (Rai, 2012). Following methods can be used as per requirement:

I. **Chaptalization:** Chaptalization is another term used to imply addition of sugar only. Addition of sugar is supposed to produce substandard wine and is prohibited in some countries. In cooler climates, grapes often do not contain enough sugars to produce a balanced wine. This may be addressed by chaptalization, the addition of sucrose to the

must or the juice in the early stages of fermentation. In some countries concentrated grape must is used instead of sugar.

II. **Gallization:** Gallization is a term used to imply addition of water and sugar prior to fermentation in order to increase alcohol content, total volume, and to decrease acidity.

III. **Acidification:** This may be necessary if the pH of the must is too high, that is, if the acidity is too low. The addition of tartaric acid, malic acid or citric acid or there mix called acid blend is the usual method employed.

IV. **De-acidification:** This may be necessary if the pH of the must is too low. It is not permitted in warmer regions of the European Union. There are a number of materials that may be used, including calcium carbonate ($CaCO_3$), potassium bicarbonate ($KHCO_3$), and potassium carbonate (K_2CO_3) (Grainger and Tattersall, 2005).

2.10.3 Must sulfiting

Sulfur dioxide (SO_2) has been used for thousands of years during winemaking as an antimicrobial and antioxidant agent. It is very effective in these roles, is readily available, and is relatively cheap and easy to use. Sulfur dioxide's main role is to prevent microbial infection of the juice and thereby prevent unwanted or spontaneous fermentations by yeasts other than that planned by the winemaker and infections by undesirable bacteria (e.g. *Acetobacter, lactobacillus*). There are three forms of sulfites in wine. Molecular sulfur dioxide and bisulfite is the form that inhibits microbes. The sulfite ion (SO_3^{2-}) is mainly responsible for preventing oxidation (Ritchie, 2010). The antiseptic and antioxidant properties of sulphurdioxide (SO_2) are taken advantage of both in connection of treatment of musts prior to fermentation and later in the winemaking process. The dosage of SO_2 usually ranges between 100 and 200 ppm. The most commonly used source of SO_2 is potassium metabisulfite (KMS). Higher concentration of SO_2 make delay fermentation (sometimes as long as 2 months) (Rai, 2012). SO_2 is added before the fermentation process to prevent air from oxidizing the juice and converting the alcohol into vinegar. The air has bacteria principally *Acetobacter* i.e. it is alive in the presence of air of oxygen. These *Acetobacter* cannot convert alcohol into vinegar because SO_2 being hungry for oxygen, takes of the oxygen from the must to let the wine yeast which in anaerobic condition convert the fruit sugar into alcohol. SO_2 also forms a coating on the surface of juice to prevent the air entering the juice (Andrew, 1980). SO_2 here seems to play a role in scavenging these side products delaying the onset of

browning. However, since SO_2 is not as effective as an anti-oxidant as AA, the by-product catalyzed browining proceeds in a competitive manner with the SO_2 at most playing a delaying role. Most authors agree if wine is packaged with sufficient SO_2 and good DO control (including ensuring that O_2 transmission through the closure is limited) that the AA levels will not decline to the point where these secondary adducts will play a role in the reasonably expected lifetime of a white wine (Wilkes, 2001).

Sulfur dioxide can react with compounds other than oxygen that may be found in musts (e.g., anthocyanin, acetaldehyde (acetaldehyde has undesirable organoleptic properties), to form `bound' SO_2, which is unable to prevent microbial spoilage or oxidation. Consequently, when we add sulfur dioxide to a juice or wine, not all will be available to protect the wine (depending on its distribution between the different forms), which complicates deciding how much to add. In practice, we have to make an estimate of how much will be in the bound form to ensure that there is sufficient molecular SO_2 (Ritchie, 2010).

2.10.4 Yeast

Wine yeasts are the member of genus of *Saccharomyces* and consequently of great individual importance (Austin, 1968). A good quality of wine yeast should have the following characters (Varnam and Sutherland, 1994):

i. Introduction of flocculation and reduction of H_2S production
ii. Reduced higher alcohol production
iii. Improved fermentation efficiency
iv. Reduced foaming.
v. Resistance to killer activity.

2.10.5 Yeast nutrition

Proper nutrient are must for the growth of yeast in cultural media. The cultural medium used must therefore contain all the essential elements for growth, in proportion similar to those occurring in yeast biomass. The elemental requirement (and the source) for yeast nutrition is given in Table 2.5.

Table 2.5 Elemental requirement and source for yeast nutrition

Element	Major source
Carbon	Sugar
Hydrogen	Water, organic compound
Oxygen	Water, dissolved oxygen, organic compound
Nitrogen	Inorganic source: NH_4Cl, $(NH_4)_2SO_4$
Phosphorus	KH_2PO_4, Na_2HPO_4
Sulphur	Na_2SO_4, $Na_2S_2O_3$ and organic sulphur compound
Potassium	KH_2PO_4
Magnesium	$MgCl_2$
Sodium	NaCl
Calcium	$CaCl_2$
Iron	$FeCl_3$, $FeSO_4$

Source: Madigan *et al.* (2000)

2.10.6 Pitch development

Within the last 20 years or so, the use of active dry yeast (ADY) in winemaking has increased considerably. It has replaced the traditional practice of yeast starters in many wineries. In this formerly widespread method, a juice is strongly sulfited (10 g/hl) to eliminate spoilage yeasts and promote the growth of wine yeasts. It is then inoculated into newly filled fermenter at a concentration of 1– 3% after several days of spontaneous fermentation. Pitch of sufficient quantity is developed before preparation of must. The developing medium should have low sugar concentration so that the 'Pasteur effect' is maintained. Pitching is done when the culture of the pitch is at its optimum stage of growth. Vigorous agitation is done after

pitching to help distribute the culture and also to help in their initial growth (Grainger and Tattersall, 2005).

2.10.7 Fermentation

Fermentation is the soul (heart) of wine making. All the desirable reactions take place during this step, so most of wine makers pay strict attention to this stage. Fermentation is the process of adding wine yeast (technically termed as *S. ellipsoidues*) to fresh juice to convert the natural sugar to ethyl alcohol. In this process, CO_2 is simultaneously released making fermentation violent at first and then slow. The yeast added is 1-3% of the volume of the juice. Generally 14 days is required for complete alcoholic fermentation. Most of the fermentation takes place in three stages.

- An initial stage during which time the yeast cells are multiplying.
- A very vigorous stage accompanied by bubbling and marked rise in temperature.
- Quiet fermentation that can proceed for quite a long time at a lower and lower rate.

Fermentation time may range from 2-20 days depending upon numerous variables- types and condition of fruits, type of wine being made, climatic condition among others. Temperature is quite critical to the fermentation process (Douglas and Considine, 1982). The optimum temperature for fermentation of Red wine is higher than that of White wine. The optimum temperature is believed to be 21.1-27.4°C (Johnson and Peterson, 1974). At temperature above 90°F (32.2°C), it is likely that wine flavor and bouquet will be injured. High temperature also encourages heat tolerant bacteria to produce acid, mannitol and off flavor (Douglas and Considine, 1982).

Johnson and Peterson (1974) reported that at the usual total sugar content of 19-24%, alcoholic fermentation proceeds rapidly and, with alcohol tolerant strains of yeast, to completion, producing about 10-12.5% alcohol (by volume). If the sugar content is greater than 24%, the high sugar content may inhibit fermentation and the rate of fermentation will be slower and may be incomplete. Under special condition of simulation, 16-18% alcohol can be reached. It is generally agreed that methanol is not produced by alcoholic fermentation, from glycine for example, but is primarily derived from hydrolysis of naturally occurring pectin. The amount of higher alcohols produced is less when ammonium

phosphate is added prior to fermentation. At very low concentration the higher alcohols may play a desirable role in sensory quality (Amerine *et al.*, 1980).

Guymon *et al.* (1961) showed that oxidative conditions during fermentation favor higher alcohol production. According to Gentillini and Cappelleri (1959), glycerol production is favored by low temperature, high tartaric content and by addition of SO_2. Most of the glycerol develops in the early stages of fermentation. Most enologists consider that glycerol is of considerable sensory importance because of its sweet taste and its oiliness. Acetaldehyde is a normal by-product of alcoholic fermentation. Kielhofer and Wurding (1960) showed that acetaldehyde retention is much greater when SO_2 is added before the fermentation. According to Kielhofer and Wurding (1960), the primary source of acetaldehyde is from enzymatic process, i.e., in the presence of yeast. Acetaldehyde reacts with ethyl alcohol to form acetal, a substance with a strong aldehyde like odor, found very little in wines (Amerine *et al.*, 1980).

The tartaric, malic and citric acids of the must are found in the resulting wines but in decreased amounts. They are important constituents of wine not only for their acid taste but also because they protect the wine from spoilage, maintain the color, and are themselves sometimes attacked by microorganisms. Malic acid disappears during alcoholic fermentation to the extent of 10 to 30%. Succinic acid is a product of alcoholic fermentation. Lactic acid has a slight odor and is a weak acid. It is a constant by-product of alcoholic fermentation, 0.04 to 0.75 g/L. Carbonic acid constitutes a very special case for both still and sparkling wines. It has no odor and very little taste. But it does have a feel and disengagement of the bubbles from the wine probably brings more oxygen away from the surface of wine (Amerine *et al.*, 1980).

The end of fermentation is signaled by a clearing of the liquid, by a vinous taste and aroma, and by a drop in temperature, and can be confirmed by checking degrees balling (sugar residual) (Douglas and Considine, 1982).

2.10.8 Racking

After completion of fermentation, the wine must be separated from the dead cells because, it may lead to yeast autolysis and, at low redox potential, formation of H_2S which give off flavors and odors to wine. This dead yeast settle at the bottom of the fermentation vessel and the wine is carefully transferred (siphoned) to other vessel without disturbing the dead yeast

leaving some wine at the bottom called lees (Andrew, 1980). Racking is the process of transferring juice or wine from one vessel to another, leaving any sediment behind. One of the most important factors in producing clear, stable wine is racking, i.e. Siphoning (Grainger and Tattersall, 2005). Normally, wine should be racked within a month of the end of fermentation. Racking process normally entails a sacrifice of 2-3% wine in lees (Rai, 2009).

The advantages of racking are (Andrew, 1980):

i. It helps removing CO_2.
ii. It raises O/R potential, which retards the formation of H_2S.
iii. It clarifies the wine.

2.10.9 Fining and filtration

Fining is a process of converting cloudy wine into clear wine. With the coarse sediment removed by racking or centrifuge, there remains other lighter matter suspended in the wine known as colloids. These are capable of passing through any filter. If not removed they will cause the wine to look 'hazy' and then form a deposit. The colloids are electrostatically charged and can be removed by adding another colloid with the opposite charge. Examples of such fining agents are egg whites, gelatin, isinglass (obtained from swim bladders of fish) and bentonite. Quantities need to be carefully controlled otherwise the fining agent itself will form a deposit, or a further, opposite, electric charge may be created. Fining may also be used to remove excess tannin and so improve the taste of the wine. Phenolic compounds are absorbed by the substance PVPP (polyvinyl polypyrolidone). This may be used at the fining stage to remove color from white wines and help prevent browning (Grainger and Tattersall, 2005).Typically, bentonite can be used at a rate of 1.5 g/L. However, it is essential that the fining agents be tested for dosage optimization before use because, over fining can cause a permanently cloudy wine (Rai, 2009).

Filtration is the process used to remove solid particles, and may take place at various stages in wine making, for example must or lees filtration. However, one of its main uses is in the preparation for bottling. The processes of fining and filtration are not interchangeable. There are three principal categories of filtration, which may be used at different stages in the winemaking process.

1. **Earth filtration**

This filtration method is used for initial rough filtration and can remove large quantities of 'gummy' solids, which consist of dead yeast cells and other matter from the grapes. The filtration takes place in two stages. Firstly, a coarse grade earth called Kieselguhr, which is commonly used as the filter medium, is deposited on a supporting screen within a filter tank. A mixture of water and Kieselguhr may be used to develop the filter bed. This is known as percolating. Secondly, more earth is mixed with wine to form slurry that is used continuously to replenish the filtration surface through which the wine passes. Wine is passed through the filter and the bed gradually increases in depth. Eventually it will clog and the Kieselguhr will have to be completely replaced with fresh material (Grainger and Tattersall, 2005).

2. **Filtration (plate and frame filter)**

A series of specially designed perforated steel plates are held in a frame. Sheets of filter medium (cloth or paper) are suspended between the plates, which are then squeezed together by screw or hydraulic methods. The filter sheets are available with various ranges of porosity filter aid such as hyflosupercel, diatomaceous earth etc are used to facilitate the filtration process. Wine is pumped between pairs of plates to pass through the filter sheets into a cavity in the plates and then to exit the system. Yeast cells and other matter are trapped in the fibres of the filter media (Grainger and Tattersall, 2005).

3. **Membrane filtration**

In recent years microfiltration has been increasingly applied as the final process before bottling. Microfiltration membranes are usually in a tubular configuration for use with wine. Pre-filtration is not required, but clarifying and stabilizing agents such as bentonite are still necessary to maintain a sufficiently high product flow. The capital cost of microfiltration system is relatively high, but this is offset by the operating efficiency, reliability and versatility. Maintenance and cleaning costs are also low (Varnam and Sutherland, 1994). The membrane operates as a molecular sieve which permits the passage of water, ethanol, flavor compounds, selected macromolecules and other dissolved species, but retains suspended material such as colloids and microbial cells. They also greatly reduce the number of bacteria. The process is not used for full-bodied red wines as it can reduce body and flavor (Grainger and Tattersall, 2005).

2.10.10 Stabilization of wine

Stabilization may be carried out to prevent tartrate crystals forming after the wine has been bottled. The tartrates are either potassium or calcium salts of tartaric acid and the crystal are also called *wine diamonds* and are totally harmless. They are sometimes found on the cork or as sediment in the bottle, and sometimes cause unwanted concern to consumers. To inhibit the precipitation of tartrate crystals in bottle, the wine is chilled to -4°C, or colder in the case of liqueur (fortified) wines. After approximately 8 days the crystals will have formed, and the cleared wine can be bottled. Another method of removal is to reduce the temperature of the wine to approximately 0°C and seed it with finely ground tartrates, followed by a vigorous stirring. The seeds then attract further crystals to them and the entire process of removal takes just 24 hours or so (Grainger and Tattersall, 2005).

2.10.11 Maturing and ageing of wine

This is one of the most interesting and one of the most important, yet one of the most complex processes of wine making. Newly fermented wine is cloudy, harsh in taste, yeasty in flavor and odor, and without the pleasing bouquet that develops later in its history (Rai, 2009). Maturation in winemaking terms is the time period, and associated changes, that occur in a wine between alcoholic fermentation and bottling, while the wine is still in bulk storage in the production facility. The period after bottling and before consumption in the life of a wine should be referred to as 'bottle ageing,' but for the purposes of discussion, it shall just be termed 'ageing' (Buglass *et al.*, 2011).

Immediately after fermentation, wines may taste rough and fairly unpleasant. A period of maturation is required. This period may be anything from 2 to 24 months, or longer, depending on the style of wine being made, and may include processes such as malolactic fermentation, oak coopering, racking, ageing in tanks or barrels, fining and filtration (Buglass *et al.*, 2011) . The choice of maturation vessel and the period of time depend upon the style of wine to be produced and quality and cost factors. There are many types of maturation vessels, including stainless steel vats and wooden barrels (Grainger and Tattersall, 2005).

Chemical processes during maturation and ageing include the oxidation of phenolics and other substances, formation of aldehydes and esters and hydrolysis of glycosides and other components. Physical effects include salt precipitation, loss of carbon dioxide, evaporation of volatile substance and dissolution of oak components. Effects may include loss of brightness,

changes to the color of the wine and character of the bouquet, and rounding and softening of tannins (Buglass *et al.*, 2011). Aging of wines improves the flavor and bouquet due to oxidation and formation of esters. These esters of higher acids formed during aging give the ultimate pleasing bouquet to the well-aged wine (Clarke and Bakker, 2004).

2.10.12 Bottling

Following filtration and clarification the wine passes to storage tanks prior to bottling. The use of glass bottles is universal for high quality wine. Bottles are cleaned, dried with hot air and cool for this purpose. The cork is the traditional means of closing the bottle, and this is protected from dehydration and mold growth by a lead foil or, in recent years, a plastic outer cap. Wine is bottled under an inert atmosphere (CO_2 and / or nitrogen) to protect wine from oxidation. Additions may also be made before bottling to stabilize the wine against microbiological and chemical deterioration, SO_2 and sorbic acid are most commonly used (Varnam and Sutherland, 1994).

2.10.13 Pasteurization

Pasteurization is the process used to kill microorganisms present in the wine so that fermentation is stopped and increase the shelf life. Wine pasteurization usually occurs for shorter periods or at lower temperatures than typical for products such as milk. This is possibly due to wine's low pH and ethanol content, both of which markedly depresses the thermal resistance of yeasts and bacteria. And approximately 3 min at 60℃ should be sufficient for a wine at 11% ethanol. Flash pasteurization at 80℃ usually requires only a few seconds as well hot bottling of wine at temperature 55-70℃ can also be done. Sulfur dioxide reduces still further the need for heating. High temperatures markedly increase the proportion of free SO_2 in wine. Although pasteurization kills most microbes, it does not inactivate the endospores of *Bacillus* species. On rare occasions, these bacteria may induce wine spoilage. The quality of some wine is reduced by pasteurization while that of other may be improved. Pasteurization inactivates the enzymes but injure the quality of the product due to complexities of establishing the most appropriate time and temperature conditions for pasteurization, membrane filters have replaced pasteurization in most situations. Filters also result in few physical or chemical disruptions to the sensory characteristics of wine. Membrane filters with a pore size of 0.45 μm or less are standard (Jackson, 2014).

2.10.14 Finishing

The traditional method of finishing the wine was to turn the bottles on end, place them in racks at about 45° angle and turn them to the left and right daily to get the yeast deposit into the neck of the bottle and on the cork. The process is called riddling “reumage”. The temperature of the whole bottle is then reduced to about 30°F to 40°F. The neck of the bottle containing the yeast deposit is then frozen (by placing in brine or other freezing solution) When the cork is removed the solid plug containing the yeast is ejected. This is called disgorging (Pederson, 1980).

2.10.15 Storage of wine

Storage of wine is an important consideration for wine that is being kept for long-term ageing. There are some factors that have the most direct impact on a wine's condition are temperature, light and humidity. The perfect storage temperature for wine, is supposed to be 52°F (11°C), anything between 40°F and 65°F (5°C and 18°C) will in fact suffice for most styles of wines. All wines are affected negatively by the ultraviolet end of the light spectrum, hence, in the cellar; wines are stored in corrugated boxes or wooden crates to protect the wines from direct light. Certain humidity (between 60 and 70%) is essential to keep the cork moist and flexible, thereby avoiding oxidation. The position in which a wine bottle is stored is also extremely important. Most wines should be laid horizontal position so that the wine keeps the cork moistened, and therefore fully swollen and airtight. Exceptions to this rule are sparkling wines and any wine that has been sealed with a screw top lid should also be stored under vibration-free conditions, but this only becomes a significant factor over a long period for sparkling wines and mature wines with sediment (Stevenson, 2005).

2.10.16 Yield of alcohol

The theoretical conversion of 180 g of sugar into 88 g of carbon dioxide and 92 g of ethanol means that yield of ethanol is 51.1% on a weight basis. This percentage may vary depending upon inoculum size, fermentation temperature and nutrient availability (Usansa, 2003). Under special condition of simulation 16-18% alcohol can be reached, but normally in commercial operation, 13-15% is the maximum (Johnson and Peterson, 1974).

2.11 Nutritional aspects and health benefits of wine

The excessive abuse of distilled alcoholic beverages, combined with religious and political conservatism, created a backlash against all beverages containing alcohol. From a scientific standpoint, much more attention has been given by the researchers to the non-nutritional aspects of wine than to what substances, in addition to alcohol, it may contain of tangible value to the consumer. Now, research concentration has largely been directed to better understanding such aspects as flavor, bouquet, keeping qualities, better ways to utilize, chemistry and biochemistry etc. in processing (Douglas and Considine, 1982).

According to Louis Pasteur, wine is the "healthiest and most health-giving of drinks." The use of wine as a medicine, or as a carrier for medications, has a long history. It goes back at least to the ancient Egyptians. Ancient Greek and Roman society used wine extensively in herbal infusions (Jackson, 2000).

According to Mmegwa (1987) beer and wine contain some nutrients present in the original malted barley and the fruit juice used in their proportion and naturally their energy value would be higher than that of distilled liquor; 100 ml of wine gives about 80 Kcal. Wine's major nutritional value comes from the rapidly metabolized, caloric value of its ethanol content. Alcohol does not need to be digested, and can be absorbed directly through the intestinal wall. In rural viticultural areas, wine historically functioned as a major source of metabolic energy for the adult population. Wine in those regions was a food (Jackson, 2000).

Wine contains small quantities of several vitamins, notably the B vitamins, such as B_1 (thiamine), B_2 (riboflavin), and B_{12} (cobalamin). Morgan *et al.* (1939) reported that about $2/3^{rd}$ of the thiamin and riboflavin in grape juice is lost during winemaking but that very little is lost during aging. They found that white wines contained more riboflavin as well as, the mineral contents of red wine generally exceed those of white wine, notably as regards potassium, sodium, phosphorus, magnesium, iron, strontium, manganese, zinc, copper, barium, and thus in terms of total ash. Red wines were slightly lower in calcium and Aluminium. As regards to vitamin content of wine, Lucia (1954) reported that when wines are taken along with a good and balanced diet, their content of thiamine, riboflavin pentothenate, niacin and vitamin B_6 contribute to total nutrition. Although wine contains soluble dietary fiber, especially red wines. It is insufficient to contribute significantly to the daily recommended fiber content in the human diet (Jackson, 2000).

Nowadays, it is becoming equally clear that moderate wine consumption (250– 300 ml/day) has undeniable health benefits. Multiple epidemiological studies suggest that daily, moderate, alcohol consumption and especially wine is associated with a reduction in all-cause mortality. This is expressed in a U-shaped curve, with increased mortality being associated with both excess alcohol intake and abstinence. This is particularly evident in the reduced incidence of cardiovascular disease in moderate alcohol consumers. In addition, it reduces the likelihood of non-insulin dependent diabetes, combats hypertension, and reduces the frequency of certain cancers and several other diseases. These epidemiological correlations are being supported by *in vivo* studies that provide molecular explanations for these associations. Wine also has several indirect benefits on food digestion. wine stimulates the production of gastric juices and foster a healthy appetite (Jackson, 2014).

2.12 Herbal wines

Wine is used as a base for medicinal preparations compounded with various herbs to treat specific diseases and disorders right from start of this century. Regular, but limited ingestion of these herbal wines tend in minimizing the need for synthesizing medicines for treating various disorders by getting benefits of the herbal extracts. Numerous herbs are used to prepare herbal wine like amla, tulsi, ginger, *Aloe vera*, tea, peppermint, and lemongrass where either the herb is used solely as substrate or combinations of herbs are used or fruit juice is used as substrate (orange or apple juice). The combinations gave a novel product with better qualities, increased acceptability and wider applications. So, such fortifications need to be explored for developing products that could be included in the realm of health (Rathi, 2018).

2.12.1 Herbs

Botanically, herbs are soft-stemmed plants, the main stem of which dies to the ground level and either does not regrow (annuals), grows again the following year only (biennials) or regrows each year (perennials). Culinary herbs are a restricted group of such plants together with some traditionally used non-herbs such as sage (sub-shurb) and bay laure (the level of large tree). All have been used for centuries in the seasoning of foods as well as many other domestic and commercial outlets. Herbs may be used fresh or after dehydration. In the latter case, it is usual to separate the leaves, floral parts and seeds from heavier, harder and less aromatic stems by screening. Such herbs are called broken or rubbed, and it is in this form

that they are generally sold for domestic and commercial use. Herbs, for the most part have a light and very distinct aromatic character although they contain relatively low levels of essential oil (Reineccius, 2006).

Herbs are usually used in foods, for making medicines, for pest control, and also for spiritual purposes. Since ancient times, the culinary and medicinal values of different herbs have been appreciated by almost every part of the world and among different cultures. They can be classified into innumerable categories depending on their scientific family and genus (Patil, 2019) .

The use of herbs in the flavouring of foods is almost as old as man himself. Herbs have tremendous importance in the way we live, as a ingredients in food, alcoholic beverages, medcicine, perfumery, cosmetics, coloring, and also as garden plants. Herbs are used in foods to impart flavor, pungency and color. They also have antioxidant, antimicrobial, pharmaceutical and nutritional properties. In addition to the known direct effects, the use of these plants can also lead to complex secondary effects such as salt reduction, improvement of texture and prevention of food spoilage. They also form an important component in quite a few alcoholic beverages and beers. Herbs are rich in volatile oils, which give pleasurable aromas. In addition, herbs may contain alkaloids and glycosides, which are of greater interest to pharmacologists (Peter and Babu, 2004).

According to Peter and Babu (2004), different parts of herbs (such as roots, buds, flowers, fruits, barks, leaves or seeds) are used. Only little can be said in a general way about their composition. Some of the main active constituents in herbs are as follows:

a. Acids-sour, often antiseptics and cleansing.
b. Alkaloids- bitter, often based on alkaline nitrogenous compounds. They affect the central nervous system and many are toxic and addictive.
c. Anthraquinones- bitter, irritant and laxatives, also act as dyes.
d. Bitters- various compounds, mainly irridoides and sesquiterpenes with a bitter taste that increase and improve digestion.
e. Coumarines- antibacterial, anticoagulants, with a smell of new-mown hay.
f. Flavones- bitter or sweet, often diuretic, antiseptic, antispasmodic and anti-inflammatory. They are typically yellow and present in most plants.
g. Gums and mucilage- bland, sticky or slimy, smoothing and softening.

h. Resins- acrid, astringent, antiseptic, healing. They are often found as oleoresins or oleo-gum resins.
i. Saponins- sweet, stimulant hormonal, often anti-inflammatory or diuretic, soapy in water.
j. Tannins- astringent, often antiseptic, checking bleeding and discharges.
k. Volatile oils- aromatic, antiseptic, Fungicidal, irritant and stimulant.
l. Glycosides- there are main four main kinds, viz.
    i. cardiac: affecting heart contractions
    ii. synogenic: bitter, antispasmodic sedative, affecting respiration and heart rate
    iii. mustard oil: acrid, extremely irritant and
    iv. Sulphur: acrid, stimulant, antibiotic.

Plants play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In this way indigenous medicinal plants play significant role of an economy of a country. Plants, as extracts and in various other forms, are being used for centuries in different traditional system of medicine for the treatment of human ailments, particularly those caused by pathogenic bacteria, fungi, as well as virus (Ray *et al.*, 2004).

Presence of various compounds and their uses has extensively been emphasized by number of workers. Progress in medicinal plant research has undergone a phenomenal growth during last two decades. Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of medicinal plants as antitumor, antianalgesic, insecticides, rotenoides etc. Demand on plant based therapeutics has increased many fold because they are natural products having no side effects and easily available at affordable prices (Verma *et al.*, 2010).

Some herbs used in herbal wine preparation are given in Table 2.6.

Table 2.6 Herbs used in herbal wine preparations

English name	Botanical name	Family	Parts used	Quantity (g/kg)	Medicinal properties
Ink nut	*Terminalia chebula*	Combretaceae	Fruit	5	Antiasthmatic, antidysenteric, antiparalytic
Belliric myrobalan	*Terminalia belerica*	Combretaceae	Fruit	5	Anthelmintic, antiseptic, astringent, expectorant, laxative, lithotriptic, rejuvenative
Indian gooseberry	*Emblica officinalis*	Phyllantheae	Fruit	5	Diuretic, laxative, carminative, stomachic, astringent, antidiarrheal, antihemorrhagic and antianemic
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome	3	Anti-dyspepsia and anticancer
Bael	*Aegle marmelos*	Rutaceae	Fruit	5	Anti-chronic diarrhea, antidysentery,

Bishop's weed	*Carum copticum*	Apiaceae	Fruit	3	Antispasmodic, antiseptic
Holy basil	*Ocimum sanctum* L.	Lamiaceae	Leaves	5	Anti-bronchial, antiasthmatic, antimalarial, antidysenteric, antiarthritis
Night jasmine	*Nyctanthes arbortristis*	Oleaceae	Leaves	2	Antisciatica and antiarthritic, antipyretic and ulcerogenic activity
Malabar nut	*Justcia adhatoda*	Acanthaceae	Leaves	2	Antispasmodic, bronchodilator and mucolytic agent in asthma
Five-leaved chaste tree	*Vitex negundo*	Lamiaceae	Leaves	2	Anti-inflammatory, antipyretic, tranquilizer, bronchial smooth muscle relaxant, antiarthritic, anthelminthic and vermifuge
Garlic	*Allium sativum*	Amaryllidaceae	Fruit	3	antioxidant,, anticancerous

Cardamom	*Elleteria cardamomum*	Zingiberaceae	Fruit	2	Antiasthma, anesthetic in burning sensation, anti- cold and cough, protects from diseases of bladder and kidney
Cinnamon	*Cinnamonum verum*	Lauraceae	Fruit	2	Anti-flatulence, anti-piles, anti-amenorrheal, antidiarrheal
Indian aloe	*Aloe vera*	Xanthorrhoeaceae	Leaves	2	Anesthetize tissues, acts against bacterial, fungal and viral Growth,anti-inflammatory, dilate capillaries, anticancer and enhance blood flow
Belladonna	*Atropa belladona*	Solanaceae	Root	2	Promoter for wound healing
Asparagus	*Asparagus adscendens*	Liliaceae	Root	2	Anti-spermatorrhea, leucorrhea, antidiarrhea

Round leaf buchu	*Barosma betulina*	Rutaceae	Leaves	2	Stimulates kidney, cleanses blood, antiseptic, acts as tonic and promotes sweating
Neem	*Azadirachta indica*	Meliaceae	Bark	0.5	Analgesic, alternative, curative of fever

Source: Panda (2012)

2.12.2 Health benefits of herbs

Patil (2019) has mentioned the following health benefits of herbs:

- **Strengthen the immune system**

Herbs are rich in many essential oils, antioxidants, phytosterols, vitamins, and other nutrient substances that equip the body to fight against toxins and germs, as well as boosting the immune system. Some of these immune-boosting herbs are elderberry, garlic, ginger, onion, hibiscus, cinnamon, and goldenseal.

- **Anti-inflammatory properties**

The essential oils present in some herbs, like ginger root, have excellent anti-inflammatory properties. Some herbs inhibit the enzyme cyclooxygenase (COX), which facilitates inflammatory reactions in your body.

- **Reduce blood sugar and cholesterol levels**

Some herbs have positive effects on the pancreas, thereby balancing blood sugar levels. Herbs like psyllium, fenugreek, and licorice can result in a noteworthy reduction of cholesterol and in blood pressure levels, thereby preventing various coronary ailments.

- **Prevent Alzheimer's disease**

These can effectively prevent Alzheimer's disease. In Europe, the Ginko herb has been used widely to treat Alzheimer's disease and other forms of dementia.

- **Prevent cancer**

Since ancient times, especially in Chinese medicine, herbs were extensively used for treating cancer symptoms. Researchers at Memorial Sloan Kettering Cancer Center have shown through a number of studies that gastric, hepatoma, colon, and breast cancer cells can be effectively destroyed by many medicinal herbs like oldenlandia, scutellaria, taraxacum, and phragmites. The herbs purify blood and prevent cell mutations that usually lead to cancerous growths.

- **Skin care**

Amongst the innumerable herbs found all over the globe, some common herbs like neem, turmeric, *Aloe Vera* and basil assure radiant and healthy skin.

- **Hair care**

Massaging your hair with jojoba oil stimulates bountiful growth to hair. There are many more herbs like gotu kola, horsetail, ginseng, and marigold extract that similarly stimulate hair growth.

- **Dental care**

There are numerous herbs that, when used directly on the teeth and gums, give wonderful results. For a healthy mouth and gums, herbs like alpine strawberry, lavender, thyme, sage, neem, fennel, parsely, *Aloe vera*, and mint are found to be very effective and are widely used in the manufacturing of herbal toothpaste, mouthwashes, and tooth whiteners.

2.12.3 General method of herbal wine preparation

The ingredients in herbal wines are up to the imagination of the maker, though there are two general paths to follow in terms of the process of making it: Either a dry-herb infusion or a fresh-herb infusion. The former infuses at room temperature and for a longer period of time, while the latter infuses in the refrigerator for 24 h. A fresh infusion is kin to sangria relying on fruit and aromatics to brighten and emphasize the herbal additions (Payne, 2016).

The exact composition of a herbal product is influenced by the method of extraction. A hot water extract is generally rich in polar components because water is a polar solvent. Oil on the other hand is a non-polar solvent and it absorbs nonpolar compounds. Alcohol lies somewhere in between the polar and non-polar compounds. Herbal wines include the alcoholic extract of herbs; usually within ethanol 12-38%. The ancient biomolecular and archaeological evidence for plant additives in fermented beverages dates from the early Neolithic period in China and the Middle East , besides from Monte Verde in Chile, around 13,000 B.P (Soni *et al.*, 2009).

A simple and quick method of preparing herb wines of many varieties is by the use of a standard basic recipe, such as barley wine, with the addition of the necessary herbs or the herb flavour extracted into the water for making the wine by steeping the dried herbs or boiling the fresh herbs. Particular attention should be paid to obtaining the maximum extraction of the flavors and qualities of the herbs. 56.69 g of dried herb usually suffice (a standard proprietary pack costing a few shillings will meet your requirements) and certain herbs with strong aromatic qualities may be suspended in a linen bag for a few days in the liquor made from a standard basic recipe. Check from time to time until the strength of flavour is to your liking. An ordinary barley wine is an excellent base; so is a tea wine.

The herbs powdered or bruised, can be either:

- Boiled or soaked in the gallon of water and strained before adding to the main recipe.
- Be added powdered or bruised to the must; or
- Suspended in a linen bag in the fermenting standard basic must.

Another new development is the introductions of flavourings, which can be used to produce from any *finished,* wine several quite different aperitifs, and French or Italian Vermouth. Flavourings are also obtainable to produce liqueurs at home—cherry brandy, curacao, green and yellow convent, kirsch, eau-de-vie, juniper gin, etc (Berry, 1996).Well-defined classes of extraction are described in the official pharmacopoeias and these may be used singly or in combination, depending on the desired outcome.

- Maceration and expression
- Digestion
- Percloation
- Infusion and Decoction

- **Maceration**

Maceration is the process of softening plant material by soaking, facilitating the dissolution of the soluble constituents. It plays an important role in many official preparations, including tinctures, extracts, syrups, wines, and vinegars. Macerations differ from water-based infusions and decoctions on the following respects:

1. The menstruum is usually alcohol.
2. The herb remains in contact with the menstruum for a longer period of time
3. The process is conducted at ordinary temperature.
4. After starting, the liquid left in the herbal material (the marc) is pressed out and mixed with the strained liquid.

The specified amount of herb, in the specified form (for example, cut or powdered), is placed with the required amount of menstruum into a vessel. The vessel is closed, to prevent the loss of alcohol, and then shaken so as to turn the contents, preferably on a daily basis. The shaking displays the saturated layer of menstruum that surrounds the herb particles and allows fresh liquid to come into contact with the herb. Actual maceration time will depend on the specific herb, but 7 to 14 days is a good general rule of thumb.

After the prescribed amount of time, the liquid is drained from the marc. The marc is then pressed to retrieve more of the menstruum. The expressed liquid is mixed with strained liquid, and the mixture is left to stand until it is clear, after which is filtered (Hoffmann, 2003).

- **Digestion**

Digestion is a form of maceration that involves application of a gentle heat ta the substance being extracted. It is used in cases in which a moderately elevated temperature will help increase solvent powers of the menstruum. Digestion differs from decoction in that preparations made through the process of digestion are alcohol based, rather than water based (Hoffmann, 2003).

- **Percolation**

Percolation is a process by which a powdered contained in a suitable vessel is deprived of its soluble constituents by descent of a solvent through the material (Hoffmann, 2003).

- **Infusion**

An infusion is a water-based preparation made by steeping leaves, flowers, and other nonwoody plant parts in either hot or cold water. Obviously, this method of preparation is appropriate only for herbs with water-soluble constituents (Hoffmann, 2003).

- **Decoction**

A decoction is water-based preparation made by gently simmering the herb in boiling water. Decoction differs from the infusion in that it is more appropriate for tougher plant parts, such as roots, bark, and seeds (Hoffmann, 2003).

A process flow chart for making herbal wine from purple sweet potato given by Panda (2012) is shown in Fig 2.4.

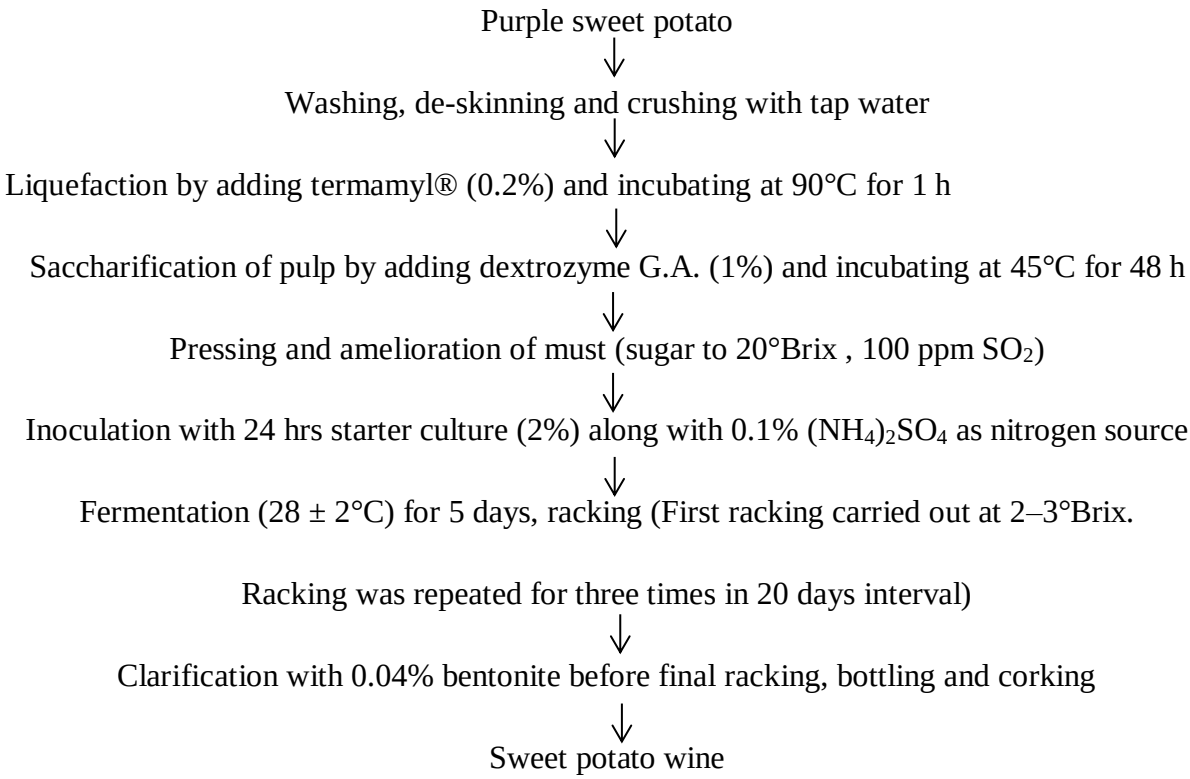

Fig 2.4 A flow chart for making anthocyanin-rich sweet potato wine.

2.12.4 Household methods of preparing herbal wines

Some common household methods of preparing herbal wines as described by Payne (2016) are as follows:

I. Blackberry-Hibiscus Wine

Ingredients

Water, sugar, blackberries, dried hibiscus, campden tablets, dry wine yeast, yeast nutrient, a pinch of tannin.

Procedure

- Sanitize a gallon bucket, lid, airlock and spoon.
- In a stockpot, bring water to a boil and stir in sugar until it dissolves, then add hibiscus and remove from heat. Let it cool, and then pour through a sterilized strainer into a bucket.
- Place blackberries in a mesh bag in the bucket. With clean hands, mash the bag so that the blackberry juice is extracted into the liquid.
- Crush campden tablet and stir into the liquid. Add the lid and attach the airlock, then let sit for a day.
- Prepare the yeast starter by scooping out one cup of the liquid with a sterilized measuring cup into a canning jar. Add the yeast and cover the jar with plastic wrap secured with a rubber band. Shake well and let stand for about three hours.
- Add yeast starter, tannin, and yeast nutrient and stir vigorously, then replace the lid and airlock. Repeat the stirring for seven days using a sterilized spoon and then ferment till completion.

II. **Juniper wine**

Ingredients

Sprig of fresh or dried juniper (or rosemary), juniper berries, **c**itrus peel, dried, cut and sifted, teaspoons coriander seeds, allspice berry, small bay leaf, dried and torn, cups dry sherry.

Procedure

- Combine all the ingredients in a clean, pint-size mason jar.
- Gently shake the contents every few days for 2 weeks.
- Keep the infusion out of direct light and away from exposure to heat.
- When ready to strain, sterilize the jar or bottle that will hold the finished herbal wine by boiling it for 10 min; do not dry.
- Line a strainer with a few layers of cheesecloth or fine-weave muslin to strain into the prepared bottle.
- Cap tightly and store at room temperature for up to 6 months for best flavor.

2.12.5 Herbal wine and health

Wine, an alcoholic drink is widely accepted, consumed, and preferred due to its nutritive and healthful properties. To enhance the basic qualities of wine, it can be fortified with certain additives that are potentially beneficial to health. When blending the herbal extracts with the wine, the mixing is done with a view to elevate the properties of the original wine and avoid undesirable changes (Rohan Shiradhonkar *et al.*, 2014). Antioxidants can be found in all part of plants such as fruits, flowers, leaf, stem and root. Therefore wines making from herbs are enrich with natural antioxidants (Yuwa-Amornpitak *et al.*, 2012) Frankel has shown that no matter how much vitamin E you take, its anti-oxidant activity plateaus at 20%, whereas wine's antioxidants will plateau at 100% after a couple glasses.

One of the main functions of anti-oxidants is to inhibit low density lipoprotein (LDL) or bad cholesterol from entering blood vessel walls and forming atheromatous plaques which eventually block off arteries causing vascular disease such as heart attack and stroke. The other main function of antioxidants is to inhibit the action of free radicals, which are negatively charged rogue molecules (with one unpaired electron in their outer orbit). The body is continuously producing waste products from its many complex biochemical pathways. These waste products include free radicals, which become free agents causing biochemical havoc leading to such things as body degeneration, aging and cancer. Wine is man's oldest medicine, having been used as such by the medical profession for over 5,000 years (Norrie, 2005).

Medicinal wines are mostly used during the winter months and also by older patients. In older patients, spleen and stomach function is typically weak and taking one's medicine as a medicated wine helps improve this situation. Likewise, older patients commonly have poor circulation. On the one hand, this means they often have cold feet or cold hands and feet. On the other hand, their diseases are often complicated by an element of stasis stagnation which a little alcohol helps address. For instance a large number of medicinal wines are for the treatment of rheumatism and arthritis in the elderly. Taking medicinal wines is very good for such conditions (Nunn, 1996).

In addition, many chronic conditions require the taking of medicine over a prolonged period of time. Cooking and taking decoctions in and out can therefore become an onerous chore, whereas, taking a nip of medicinal wine is quick, easy and enjoyable. Because a little alcohol is good for the elderly's digestion, especially in the winter, medicinal wines may also be easier on the stomach and easier to digest than medicated pills and powders.

Further, medicinal wines are typically more concentrated and potent than decoctions and pills. Therefore, they are especially useful for treating post-stroke patients whose ability to drink large quantities of liquids may be impaired (Nunn, 1996).

Health benefits from consuming herbal wines as described by Norrie (2005) are as follows:

- Reduction of Vascular Disease (due to greatly improved blood flow) resulting in
- Reduced coronary heart disease, reduced ischemic stroke, reduced deep vein thrombosis, reduced osteoporosis, increased intellect in the elderly, reduced macular degeneration (a common cause of blindness), reduced renal failure
- Tonic – wine contains many substances including most vitamins, minerals and trace elements
- Fat and cholesterol free source of carbohydrate
- Reduced cancer
- Reduced blood pressure
- Antiseptic due to alcohol and more importantly polyphenols
- Increases morale and appetite – nursing home and hospital patients
- Wine contains quercetin, resveratrol and epicatechin, which are potent anti-oxidants and also act as anti-carcinogens

- Reduction in colds
- Diabetes – dry wine only alcoholic drink that is allowed with diabetes as all the sugar has been converted to alcohol.
- Reduced gallstones
- Reduced kidney stones
- Reduced Alzheimer's disease
- Reduced Parkinson's disease
- Improved digestion
- Reduced *H. pylori* infection of the stomach and duodenum leading to reduced ulcers
- Improved physical condition of the elderly
- Reduced Hepatitis A
- Reduced stress and depression

2.12.6 Present context of herbal wine

Herbal preparations have been known to treat various infectious diseases throughout the history of mankind. Wine serves as a base for medicinal preparations compounded with a range of herbs adapted to treat various disorders. Functional botanical ingredients are more admired than ever in the beverage market. Many wines are made from herbs with perceived medicinal value and such wines have many additional health benefits. There are hundreds of beneficial compounds contained in these herbs which can deliver antimicrobial, antioxidant, anti-inflammatory, anti-mutagenic properties to the final food product (Trivedi *et al.*, 2015).

A number of studies have been reported on medicinal herbs used for production of wine either as substrate (raw material) or as adjuncts. Jamwal *et al.* (1959) reported fermentation of Indian gooseberry, also known as *Amla* (*Emblica officinalis*) fruits (having medicinal properties like diuretic, laxative, carminative, stomachic, astringent, antidiarrheal, antihemorrhagic and antianemic) to wine. Several value added products have been prepared from Amla. Amla berries can be used as a valuable ingredient for the production of an herbal beverage. Tea (*Cammellia sinensis*) leaves have been fermented to wine, having very good acceptance both on sensory and health attributes (Aroyeun *et al*. 2005).

Aloe vera known to be a panacea has been fermented to wine. *Aloe vera* (Aloe barbadensis Miller), a well-known herbal plant has been demonstrated to possess strong antibacterial, anti-inflammatory, anti-tumor and immune stimulatory properties. It has been utilized

extensively in the preparation of health drinks (Christaki and Florou-Paneri, 2010). A herbal wine from *Aloe vera* gel was prepared and its effect against common food borne pathogens and probiotics was evaluated (Trivedi *et al.*, 2012). The wine was found to be similar to any other wine in terms of its composition, and sensory quantities and exhibited bactericidal activity against common food borne pathogens (*Salmonella typhimurium*, *Staphylococcus aureus and Escherichia coli*). Its bactericidal effect was much faster in case of Salmonella as compared to other pathogens. The wine was not inhibitory to the selected probiotic strains and no significant difference in the viable count of lactobacilli was found in the fecal matter, hence, indicating their persistence in the gut of wine fed animal.

Panda (2012) prepared a herbal purple sweet potato wine using purple-fleshed sweet potato and 18 medicinal plant parts fruits of ink nut, Indian gooseberry, garlic cinnamon, leaves of holy basil, night jasmine, malabar nut, roots of belladonna, asparagus, rhizome of ginger, etc.) by fermenting with wine yeast, *Saccharomyces cerevisiae*. The starch present in PSP was enzymatically saccharified (using commercial thermostable enzymes termamyl [0.2%] and dextrozyme GA [1%]) to fermentable sugars, and the homogenized medicinal plant parts were mixed to it at desirable quantities before subjected to fermentation. The herbal wine had the following compositions: total soluble sugar (TSS), 4.0°Brix; starch, 0.24 g/100 ml; total sugar (TS), 0.95 g/100 mL; reducing sugar, 0.38 g/100 ml; titratable acidity (TA), 1.25 g tartaric acid/100 mL; phenol, 0.19 g (caffeic acid equivalent)/100 ml; anthocyanin, 59.90 mg/100 ml; lactic acid (LA), 1.92 mg/100 ml; ethanol, 8.61%, v/v; and pH 3.34. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of the wine was 51.35% at a dose of 250 µg/ml. The herbal wine thus prepared was presumed to contain the therapeutic and antioxidant properties of PSP as well as those of medicinal plant parts added as adjuncts. Herbal purple sweet potato (PSP) wine prepared from anthocyanin-rich PSP and 18 different types of medicinal plant parts was reported to be a novel and unique product with ethanol content of 8.61% v/v. The wine was rich in antioxidants such as anthocyanin and phenols and presumably possessed biological active ingredients as remedies for common ailments like cold, cough, skin diseases and dysentery.

Lee *et al.* (2013) prepared 'Fuji' apple (*Malus domestica*) wines containing pine (*Pinus densiflora* Siebold et Zuccarini) needle and *hwanggi* (*Radix Astragali*)/mistletoe (*Viscum album*). Normal apple wine was fermented rapidly, but after 40 days fermentation/maturation, the final ethanol content, pH, total acidity, and contents of sugar/organic acid showed similar

levels in three kinds of apple wines. The total phenols and antocyanins contents, and brightness were higher in apple-pine wine and apple-herb wine than in normal apple wine. Apple-herb wine had higher values in total phenol contents, brightness, free amino acid contents, and quenching activity for ABTS free radicals than normal apple wine, and had similar sensory evaluation values with normal apple wine. It is supposed that *hwanggi* and mistletoe might provide functional components to normal apple wine and might be applied to development of functional apple wines.

2.13 Wine analysis

Throughout the history of wine making, analytical techniques have become increasingly important with the development of technology and increased governmental regulation. Analysis of wine is performed for a number of reasons such as quality control, spoilage reduction and process improvement, blending, export certification and global regulatory requirements (Fugelsang, 1996).

2.13.1 Physical and chemical analysis

All wines should be subjected to appropriate analyses during their production and storage to meet the requirements of regulatory agencies and to give the winemaker information to monitor the operations properly (Fugelsang, 1996).

Experimental wines often require additional analyses to obtain more complete information and study the specific effects of the experimental conditions. There is no sense in doing the experiments unless analytical methods are available to evaluate the results. Planning for these analyses and the labor and timing for them should precede initiation of the experiments. Some analyses can be done more or less at leisure on the finished wine, others must be done at specific moments or the experiment is spoiled. Sometimes interim samples can be quickly frozen and held for later analyses as a group. Other cases arise where this is not possible for experimental or logic reasons (Boulton, 1998).The components of wine and must can be broken into classes and are given in Table 2.7.

Table 2.7 Components of wine.

Soluble solids:	sugar extract glucose and fructose
Acidity:	total volatile pH individual acids
Alcohols:	ethanol methanol fusel oils glycerol
Carbonyl compounds:	acetaldehyde HMF diacetyl
Esters:	ethyl acetate methyl anthranilate (labruscana)
Nitrogen compounds:	NH_3 amino acids Amines proteins
Phenolic compounds:	total phenolic fractions including anthocyanins
Chemical additions:	SO_2 sorbic and benzoic acids illegals
Other:	common and trace metals, oxygen, CO_2, fluoride

Source: Fugelsang (1996)

According to Amerine *et al.* (1980), the different parameters viz. alcohol by volume,(%), alcohol, glycerol, ash, total acids, volatile acids, reducing sugars, proteins, tannins and specific gravity of different wines were analyzed. According to Pearson (1981), analytical parameters of different wines were specific gravity, alcohol (g/100 ml), % total solids, % free volatile acids (as acetic acid), % fixed acid (as acetic acid), % ash and % sugar.

Different dissertations related to wine held in Central Campus of Technology, Hattisar, Dharan have mostly analyzed the parameters such as pH, TSS, alcohol content, acidity, reducing sugar, aldehydes, esters, specific gravity, total sugars, ash, methanol and higher alcohols (Raut, 2014).

2.13.2 Sensory evaluation

2.13.2.1 Development of sensory evaluation

Sensory tests of course have been conducted for as long as there have been human beings evaluating the goodness and badness of food, water, weapons, shelters, and everything else that can be used and consumed. The rise of trading inspired slightly more formal sensory

testing. A buyer, hoping that a part would represent the whole, would test a small sample of a shipload. Sellers began to set their prices on the basis of an assessment of the quality of goods. With time, ritualistic schemes of grading wine, tea, coffee, butter, fish, and meat developed, some of which survive to this day. Grading gave rise to the professional taster and consultant to the budding industries of foods, beverages, and cosmetics in the early 1900s. A literature grew up which used the term "organoleptic testing" to denote supposedly objective measurement of sensory attributes. In reality, tests were often subjective, tasters too few, and interpretations open to prejudice. Scientists have developed sensory testing, then, very recently as a formalized, structured, and codified methodology, and they continue to develop new methods and refine existing ones (Meilgaard *et al.*, 1999).

Sensory evaluation is an integrated, multidimensional measure with three important advantages: it identifies the presence of notable differences, identifies and quantifies important sensory characteristics in a fast way, and identifies specific problems that cannot be detected by other analytical procedures. The methods that have been developed serve economic interests. Sensory testing can establish the worth of a commodity or even its very acceptability. Sensory testing evaluates alternative courses in order to select the one that optimizes value for money. The principal uses of sensory techniques are in quality control, product development, and research. They find application not only in characterization and evaluation of foods and beverages, but also in other fields such as environmental odours, personal hygiene products, diagnosis of illnesses, testing of pure chemicals, etc. The primary function of sensory testing is to conduct valid and reliable tests, which provide data on which sound decisions can be made (Meilgaard *et al.*, 1999).

2.13.2.2 Sensory evaluation of wine and its importance

Sensory evaluation has become a popular research tool in the food and beverage industries and is defined by the Institute of Food Technologists as "A scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing." Changes in product formulation may produce desirable or undesirable changes in the final product and must be assessed, analyzed, then interpreted in a meaningful way. Without the proper sensory evaluation techniques it is difficult to interpret sensory response and make logical and sound decision. Even the most sophisticated chemical analysis cannot now, and probably never will, define the subtle flavors that make one wine greater than another in the opinion of observant

consumers. That is as it should be. As a consequence, it is almost always necessary to compare wines by sensory analysis in addition to chemical and physical methods. This is true of commercial wines, but often especially so with experimental wines (Savits, 2014).

Wine is an exceedingly complex beverage, containing an estimated several hundred volatile compounds. The compounds may arise from the grape itself, through the process of crushing and enzyme action, through fermentation, and over the period of maturation. In the greater sense, there are a collection of factors contributing to the vast number of wine types and styles: climate, grape variety, stage of ripeness at harvest, winemaking techniques, and storage methods. Thus, sensory evaluation methods have been applied to study characteristics in wine related to these factors. More than 1000 compounds have been identified in grapes and wine, with individual concentrations varying considerably. Our ability to perceive compounds is dependent not only upon their presence at or above a sensory threshold concentration, but also upon their interaction with other components. The sensory properties of a particular wine therefore, are dependent upon chemical and physical effects relating to the specific matrix or composition (Savits, 2014).

In spite of opinion to the contrary by wine writers and some wine makers, one person's opinion is hardly definitive on any wine's sensory character and quality. That is not to say that one tester may not be better than another in natural ability, concentrated effort, amount of experience, and/or comparative memory. In evaluation of the sensory qualities of one or more wines a panel of testers is necessary. This panel should be as sensitive and experienced as possible, but each individual is erratic, biased, or unobservant on some occasions, hence the need for panels and statistical evaluation of the testing results (Lesschaeve, 2007).

No technique is ideal for everyone. Probably the most essential property of a serious taster is the willingness, desire, and ability to focus his or her attention on the wine's characteristics. Where tasters are unfamiliar with the characteristics of the wines to be tasted, it can familiarize the senses to the basic attributes of the wines. However, the introductory sample must be chosen with care to avoid setting an inappropriate standard and distorting expectations. It is safer to encourage tasters to cleanse their palate between each sample. In contrast, olfactory adaptation may have an advantage. For example, it may "unmask" the presence of other aromatic compounds. Most wines are best sampled in clear, tulip-shaped wine bottle. The primary exception involves sparkling wines. These are normally judged in elongated, flute-shaped glasses. They facilitate observation of the wine's effervescence. All

glasses in a tasting should be identical and filled to the same level (about one-quarter to one-third full). This permits each wine to be sampled under equivalent conditions. Between 30 and 50 ml is adequate for most tastings. Not only are small volumes economic, but they facilitate holding the glass at a steep angle (for viewing color and clarity) and permit vigorous swirling (to enhance the release of aromatics) (Jackson, 2002).

The sequence and method of wine sensory evaluation can be listed as following (Jackson, 2002):

- **Appearance:** Firstly, view each sample at 30°C to 45°C against the bright white background. Then record separately the wine's clarity (absence of haze), color (shade or tint) and depth (intensity or amount of pigment), viscosity (resistance to flow) and effervescence (notably sparkling wines).
- **Odor:** Firstly sniff each at mouth of glass before swirling and then, study and record the nature and intensity of fragrance. Now swirl the glass to promote release of the aromatic constituents from wine, smell the wine initially at the mouth and deeper into bowl. Now study and record the nature and intensity of fragrance.
- **In-mouth sensations:** Take a small (6 to 10 ml) sample into mouth. Move wine into mouth to coat all surface of the tongue checks and palate. For various taste sensations (sweet ,acid, bitter) note where they perceived, when they first detected, how long they last, and how they changes in perception and intensity. Then, concentrate on the tactile (mouth feel) sensation of astringency, prickling, body temperature and heat. Record these perceptions and how they combine with each other.
- **Finish:** concentrate on the olfactory and gustatory sensations that linger in the mouth. Compare these sensations with those previously detected. Note their character and sensations.
- **Overall quality:** After the sensory aspect have been studied individually, attention shift to the integration of their effects the wine's overall quality and finally, make and overall assessment of the pleasurableness, complexity, subtlety, elegance, power, balance and memorableness of wine.

2.13.3 Color of wine

The color of red wine is derived initially from anthocyanin pigments. The fermentation of grapes for wines has a marked effect upon the color of the product. The final color may be influenced by the SO_2 content and the alcohol content attained at the time of screening (Berg and Akiyoshi, 1962). Maximum color is attained between 3 and 6 % alcohol and the amount of color extracted increases with increasing SO_2 content up to 250 ppm. The color stability during the aging of wines was superior at the higher level of SO_2. Berg and Akiyoshi (1962) noted that non-fermented wines fortified with alcohol had much higher color retention during aging than those produced by fermentation. Wine production practices including the level of SO_2 and alcohol content have an influence on the color equilibrium between anthocyanogens and anthocyanins. Often testers associate particular colors with certain wines. Young, dry, white wines generally ranges from nearly colorless to pale straw colored. A more obvious yellow tint may suggest long maceration or maturation in oak cooperage. Sweet white wine may vary from a pale straw to yellow- gold to brown. Ascorbic acid is an effective oxygen scavenger reacting with O_2 (which would otherwise react with phenolic to produce browning) around 1700 times more quickly than SO_2 (Somers and Evan, 1997).

2.14 Side effects of wines

There are other harmful effects one can suffer from consuming wine, other than those suffered from straight abuse or excessive consumption. These harmful effects are those due to allergy and side effects in general, even with exposure to the smallest amounts of wine. Many consumers think that the main cause of allergy in wine is due to Sulphur dioxide (SO_2) (Randolph and Moss, 1980).

Sulphur dioxide is listed as 220 on the back label of the wine bottle and has been used as a preservative of foodstuffs since Roman times over 2,000 years ago. It always amuses the author when people say they are allergic to wine because of the SO_2; but when the author asks them if they eat dried fruit such as dried apricots, they say that is alright – not knowing the dried fruit, for example, contains a lot of SO_2 as a preservative! What most of these people are allergic to in wine, along with most consumers, is either the histamines or tannins, both of which come from the skin of the grape and hence are usually found more in red wines that white wines. But allergy is idiosyncratic; in other words, it is up to the individual what they are allergic to. Hence the old saying – one man's food is another man's poison.

Theoretically, one could be allergic to any one of the many thousands of components in wine and Dr.Theron G Randolph has suggested that alcoholism is a severe form of food addiction, where the patient is addicted to other components in the beverage other than the alcohol (Randolph and Moss, 1980).

2.15 Wine defects and spoilage

Like beer, wine has its defects from non-microbial causes and spoilage caused by microorganisms. Defects include those, due to metals or their salts, enzymes and agents employed in coloring the wine. Iron, for example, may produce sediment known variously on grey, black, blue or ferric casse and in white wine, it may be responsible for a white precipitation of iron phosphate termed white casse. Tin and copper and their salts have been blamed for cloudiness. White wines may be turned brown and red wines may have their color precipitated by peroxidase and oxidizing enzyme of certain molds. Gelatin used in clarifying wines, may cause cloudiness. The main role of microorganisms in winemaking is to convert grape sugars to alcohol, reduce wine acidity and contribute to aroma and flavor. They can also cause numerous unwelcome wine spoilage problems, which reduce wine quality and value. Winemaking processes include multiple stages at which microbial spoilage is likely to occur and ends up with altering the quality and hygienic status of the wine. This may render the wine unacceptable, since the spoilage can include bitterness and off flavor, and cosmetic problems such as turbidity, viscosity, sediment and film formation. The main microorganisms associated with wine spoilage are yeasts, acetic acid bacteria and lactic acid bacteria (Mojsov *et al.*, 2006).

2.15.1 Wine defect caused by yeast

Yeasts play a central role in the spoilage of beverages, mainly those high acidity and reduced water activity. The spoilage caused in wine by yeasts is important because they cause re-fermentation, ester formation, hydrogen sulphide and volatile sulphur compounds, volatile acidity, the formation of volatile phenols, mousiness, film formation, deacidification and the formation of ethyl carbamate (Mojsov *et al.*, 2006).

The yeast *Schizosaccharomyces pombe* has been associated with wine spoilage when growing in bottled wine and forming a sediment at the bottom of the bottle. The yeast *Zygosaccharomyces bailii* is one of the major wine spoilage yeasts, re-fermenting juice or wine during storage. Yeasts *Hansenula anomala, Kloekera apiculata* and *Hanseniaspora*

uvarum are associated with ester taint of faulty wines, which correlates with large amounts of acetic acid. These three species are associated with grape juice and result in spoilage at the early stages of alcoholic fermentation. The ester taint can be linked to the presence of ethyl acetate and methyl butyl acetate. Hydrogen sulphide is produced by yeasts during fermentation through the sulphate reduction pathway and has a flavor threshold of 50-80 mg/L and when exceeding this value will produce the rotten egg off flavor. One of the yeasts that can withstand the toxicity of ethanol levels and which has become the latest concern for most winemakers as a result of phenolic off flavors, is *Brettanomyces/Dekkera.* Wines typically associated with a "Bretty character" is commonly recognized by aromatic defects ranging from medicinal smells to farmyard like odors and even spicy clove like aromas (Mojsov *et al.*, 2006).

2.15.2 Wine defects caused by bacteria

Bacteria are part of the natural microbial ecosystem of wine and play an important role in winemaking by reducing wine acidity and contributing to aroma and flavor. They can cause numerous unwelcome wine spoilage problems, which reduce wine quality and value. Lactic acid and acetic acid bacteria are the main families of bacteria found in grape must and wine. (Mojsov *et al.*, 2006).

In presence of air, the aerobic acetic acid bacteria, usually *Acetobacter aceti* of *Gluconobacter oxydans,* oxidize alcohol in wine to acetic acid, an undesirable process called acetification. They also may oxidize glucose in the must to gluconic acid and may give a mousy or sweet-sour taste to the must. If the larger amounts of sugar in must or wine are fermented by the lactic acid bacteria, variable amounts of CO_2, ethanol, volatile acid and mannitol are formed depending on the particular species. Wine which have undergone changes in this manner are said to have a lactic acid flavor (Prescott and Dunn, 2004).the growth of lactobacilli produces milky cloudiness, increase lactic and acetic acid and yield CO_2. It sometimes give mousy or other disagreeable flavor and damages the color of the wine (Mojsov *et al.*, 2006).

2.15.3 Prevention of wine spoilage

Winemaking processes include multiple stages at which microbial spoilage is likely to occur. The first stage involves the fruit material to be processed and equipment to be used. One must attempt to reduce the numbers of microbes in the juice and on the equipment. This is

achieved through processing the pulp by applying food hygiene practices and following the hazard analysis critical control point (HACCP) system. The second stage of microbial spoilage may occur during fermentation because at this stage, the fruit juice contains both the natural flora of the fruit and flora that may be harboured by the wine cellar and its equipment. Traditionally, sulphur dioxide has been used to control unwanted micro-organisms during winemaking, where it is usually added to bins of machine-harvested grapes and after malolactic fermentation. Sulphur dioxide acts as both an antimicrobial agent and an antioxidant in wine. Physical removal of microorganisms through filtration of juice or wine can also be used. However, filtration typically is mainly conducted prior to bottling and hence is not used to remove microorganisms during winemaking (Mojsov *et al.*, 2006).

However best way to avoid wine spoilage is not always clear-cut. As an initial barrier, the high ethanol concentrations (up to 16% v/v), high wine acidity (pH as low as 2.9) can inhibit development of bacterial populations. Storage of wine at temperatures below 15°C might assist with minimizing the ability of bacteria to proliferate in wine, but will also delay wine maturation. To prevent microbial spoilage of the finished wine, it is important to deactivate any residual microorganisms before or after bottling. This can be accomplished by pasteurization, addition of inhibitors such as SO_2 or by filtration. The delicate flavor of some wines is harmed by heating or by adding SO_2. For these wines, filtration is preferred method of removing microorganisms (Banwart, 2004).

2.16 Raw materials for herbal wine making

2.16.1 Yacon

Yacon (*Smallanthus sonchifolius*) is a perennial herbaceous plant of the family Asteraceae, native to the Andean regions of South America. Yacon yields starchy, fruit-like roots of different shapes and sizes that are usually consumed raw and taste sweet. Their crunchy texture very much resembles that of an apple. Yacon (*Smallanthus sonchifolius*) is a perennial plant which forms sweet-tasting underground tuberous roots. The roots vary greatly in shape and size; commonly they are 15-20 cm long and 10 cm thick. They come in different colors, brown, pink, purple and cream. One plant can produce more than 10 kg of roots (Ojansivu *et al.*, 2011). Yacon root's water content usually exceeds 70% of the fresh weight while the major portion of the dry matter consists of fructooligosacharides (FOS) .It has gradually received more attention due to its abundant content of fructooligosaccharides (FOS) and

phenolic compounds. FOS content ranges from 6.4% to 70% of the dry matter (0.7% to 13.2% of the fresh weight) depending upon the specific crop and location (Caetano *et al.*, 2016).

Yacon is an Andean plant that is attracting global attention for its prebiotic advantages and benefits that are due to its high content of non-digestible oligosaccharides (NDOs), such as fructooligosaccharides and inulin, as well as phenolic compounds. Therefore, yacon's tuberous roots have been used as natural sweeteners and syrups for digestive problems, particularly for balancing the intestinal microbiota. In addition to prebiotics, yacon contains flavonoids, phenolic acids and tryptophan, which display antioxidant, anti-inflammatory, antimicrobial and anticancer activities. The phenolic compounds in yacon protects biomolecules such as DNA, lipids and proteins against damaged caused by free radicals (Delgado *et al.*, 2013).

The yacon tuber contains carotenoids that confer its yellow color (Quinteros, 2000). It also contains chlorogenic acid, ferulic acid, and caffeic acid which make the tubers susceptible to enzymatic browning reactions caused by polyphenol oxidases (PPOs). To inhibit these reactions, PPOs are inactivated by the heat or by the use of reducing agents, such as sulphites and organic acids (ascorbic, malic, citric acids) (Manrique *et al.*, 2005). Nowadays, companies have also developed novel products such as yacon syrup and yacon tea. Both products are popular among diabetics and dieters. Besides this, yacon juice treated with active carbon powder, yacon vinegar, yacon wine, chocolate cake, and yacon juice blended with peach or lemon juice, are some other products that have been developed (Granato *et al.*, 2011).

There are reports on yacon cultivation in other countries, including EUA, Europe, New Zealand and Brazil. In folk medicine, yacon tuberous roots and infusions from dried leaves are consumed by people suffering from diabetes or from various digestive or renal disorders. Its tuberous roots are consumed fresh or cooked and it has been considered a functional food because of the large amounts of fructans (i.e., inulin and fructooligosaccharides). Fructans are carbohydrates reserve which contains up to 70 fructose units linked or not to a terminal sucrose molecule, may have linear or branched structure held together by frutosil-fructose bonds. Studies have shown that the best period to harvest yacon in tropical regions is between the 31st and 35th week after cultivation, regarding the concentration of fructans and their proportion in relation to mono- and disaccharides. The yacon plants present a high hydrolytic

activity at maturation phase of the tuberous roots, contributing to the predominance of a low degree of polymerization such as FOS rather than fermentable long-term fraction fructans (Moura *et al.*, 2012).

The taxonomic classification of yacon is given in Table 2.8.

Table 2.8 Taxonomic classification of yacon

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Asterales
Family	Compositae = Asteraceae

Source: Ojansivu *et al.* (2011)

2.16.1.1 Botanical characteristics and morphology of yacon plant

Yacon, a native of the Andes closely related to the sunflower, is a vigorous, herbaceous perennial plant (family Compositae or Asteraceae sunflower family). The plant produces large tuberous fruits similar to sweet potatoes in appearance, but they have a much sweeter taste and crunchy flesh. The plants are extremely hardy and are able to grow under hot or cold conditions. Yacon grows up to a height of two meters, has large opposite sagittate leaves with serrate margins, and multiple yellow-orange flowers 3 cm in size (Polreich, 2003).

The plant is distinguished by having two kinds of tuberous fruits, a central rhizome with "eyes" for producing new stems, and multiple edible tuberous fruits radiating from the rhizome. Generally, the root system is composed of 4-20 fleshy tuberous storage roots that can reach a length of 25 cm by 10 cm diameter. The flesh color of storage roots varies considerably: white, cream, white with purple striations, purple, pink, and yellow. The tuberous root bark is brown, pink purplish, cream or ivory white and very thin (1–2 mm).

The edible tuberous fruits are crunchy like a crisp, sweet, and juicier than any pear. Stem is cylindrical or sub angular ramified in most clones, hollows at maturity, density pubescent and green to purplish colored bark. Like the sunflower, the yacon presents distributed big leaves of two even along very little ramified shafts. Lower leaves are broadly ovate and hastate or sub hastate Cannale and auriculate at base; upper and lower surface are densely pubescent, the inflorescences are terminal, composed of one to five axes each with three capitula. The color of the flower varies between yellow to bright orange, ray flowers are two or three toothed (Polreich, 2003). Tuberous fruit crops, in which tuberous fruits are formed after cessation of stem growth, seem to have a similar mechanism of tuberous fruit formation to potato (Lachman *et al.*, 2003). Yacon morphological aspects are given in Fig. 2.5.

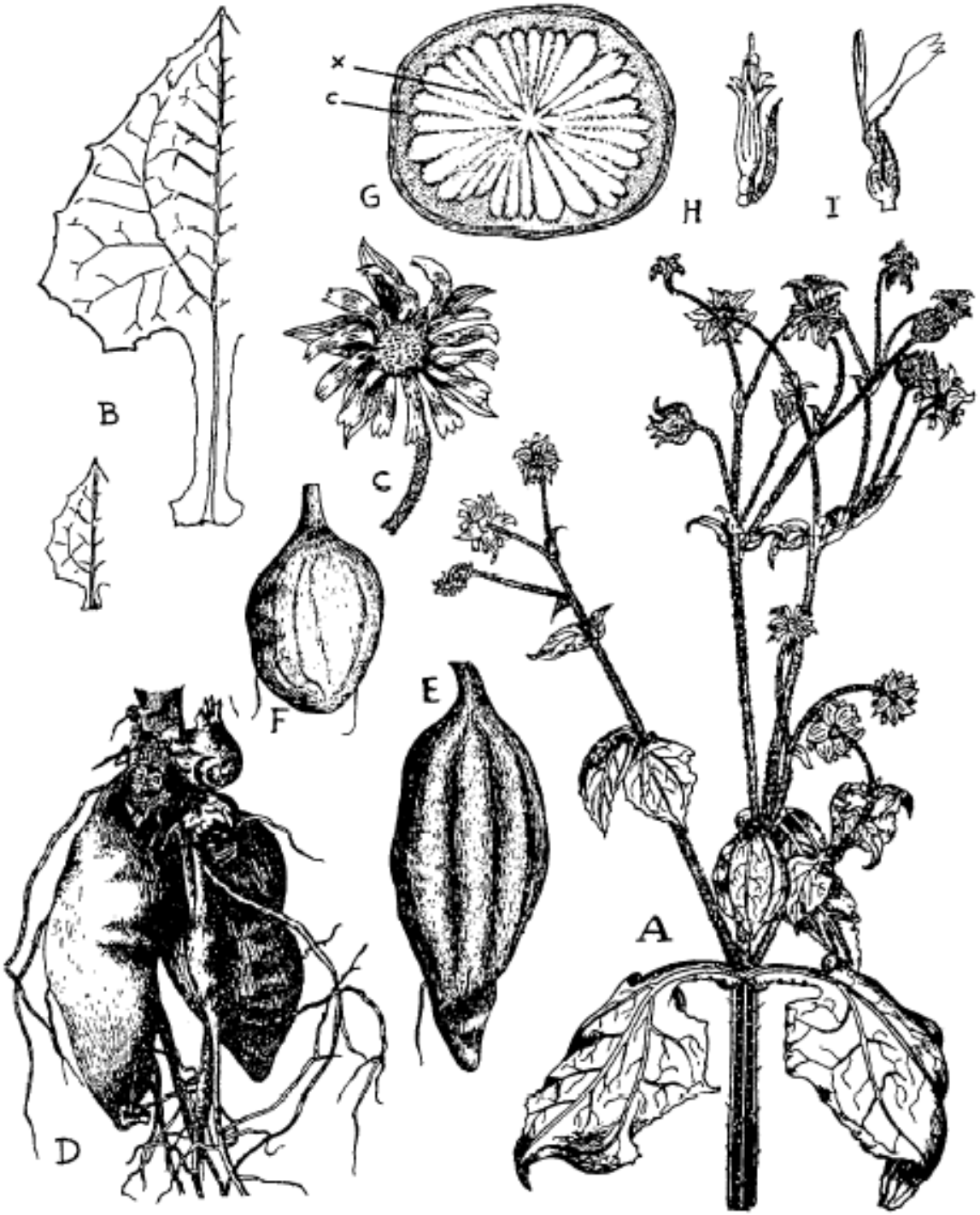

Fig 2.5 Yacon *(Smallanthus sonchifolius)* morphological aspects.

(A: flowering branches. B: leaves. C: flowerhead. D-F: tuberous roots. G: transverse section of the tuberous root (x, xylem; c, cortex tissues). H: staminate disk flower. I: pistilate ray flower.)

Source: Hermann and Heller (1997).

The Chemical composition of yacon tuber, stem and leaf is given in Table 2.9.

Table 2.9 Chemical composition of yacon tuber, stem and leaf

Compound	Part of Yacon		
	Stem	Leaf	Tuber
Water (%)	86.7	83.20	93-70
Protein (%)	1.51	2.87	0.4- 2.0
Saccharides (%)	1.55	1.44	12.5
Lipids (%)	6.30	1.24	0.1- 0.3
Ash (%)	1.35	2.68	0.3-2.0
Fibre (%)	1.51	1.68	0.3 -1.7
Calcium (mg/ 100 g)	967	1805	23
Phosphorus(mg/ 100 g)	415	543	21
Iron (mg / 100 g)	7.29	10.82	0.3
Copper (mg / 100 g)	≤0.5	≤0.5	0.963
Manganese (mg/ 100 g)	≤0.5	3.063	0.541
Zinc (mg / 100 g)	2.93	6.20	0.674

Source: Valentova and Ulrichova (2003)

- **Saccharides**

Yacon tubers contain as storage compounds mainly fructans with low glucose content. Fructans are non-digestible carbohydrates derivatives of sucrose, formed by several units of fructose with a glucose residue. They can be produced by bacteria, algae, fungi and plants. In plants fructans are used as reserves of carbohydrates, found in different organs such as leaves, roots (including yacon), tubers, rhizomes and fruits. The term fructan includes both oligosaccharides and polysaccharides. Inulin-type fructans with degree of polymerization from 2 to 10 are known as fructooligosaccharides (FOS), whereas for those with a higher polymerization degree the term most often used is inulin. FOS are chemically composed of 1 molecule of glucose connected to between 2 and 10 fructose molecules They have a favourable influence on the human intestinal flora and can modify some hyperlipidemias. Humans have no enzyme capable of hydrolysing the β (2→1) bond. B (2→1) fructans of the inulin type are thus dietary fibre or the indigestible residues of plant origin in human diet (Valentova and Ulrichova, 2003).

Recently, oligofructans have been classified as prebiotics. These are not digested in the human gastrointestinal tract and they are transported to the colon where they are fermented by selected species of gut micro-flora, especially *Bifidobacterium* and *Lactobacillus*, both indicators of a balanced gut flora. Studies have demonstrated that prebiotic consumption modifies gut flora composition and its metabolic activities. Probably through this action they also modulate lipid metabolism, calcium absorption, childhood immune systems and gut function (Valentova and Ulrichova, 2003).

The composition of saccharides in yacon tuber is given in Table 2.10.

Table 2.10 Composition of saccharides in yacon tuberous root

Saccharide	Content mg/g dry matter
Fructose	350 ± 42.0
Glucose	158.3 ± 28.6
Sucrose	74.5 ± 19.0
GF_2	60.1 ± 12.6
GF_3	47.4 ± 8.2
GF_4	33.6 ± 9.3
GF_5	20.6 ± 5.2
GF_6	15.8 ± 4.0
GF_7	12.7 ± 4.0
GF_8	9.6 ± 7.2
GF_9	6.6 ± 2.3
Inulin	13.5 ± 0.4

Source: Valentova and Ulrichova (2003)

- **Other important chemical components**

In comparison with other roots and tubers yacon contains a high level of polyphenols, which account for approximately 200 mg/100 g of fresh weight. The most abundant polyphenols are chlorogenic acid and at least four soluble phenols derived from caffeic acid. Other compounds reported with antioxidant activity are trypthophan, quercetin, ferulic acid and galic acid. Despite the high levels of polyphenols in the root, much higher levels are found in the leaves and in the stem. Polyphenols are chemical components that have antioxidant

properties. That is to say that they neutralize the oxidization caused by unstable molecules known as free radicals (Valentova and Ulrichova, 2003).

2.16.1.2 The functional effects of yacon

Yacon administered as a dietary supplement is well tolerated and produces no negative response, toxicity or adverse nutritional effects. In addition, low glucose content and high concentrations of fructooligosaccharides of yacon allow the study of possible effects in patients with metabolic diseases, such as diabetes and metabolic syndrome. In this regard, yacon has been proven to reduce levels of glucose in normal and diabetic rats with hyperglycemia (Delgado *et al.*, 2013).

Because of its inulin and FOS contents, yacon has shown important prebiotic characteristics. It resists digestion in the upper digestive tract and is hydrolyzed and fermented by colonic bacteria, such as lactobacillus and bifidobacteria. It has been shown that the association between prebiotics and probiotics has beneficial consequences for the intestinal tract. Bifidobacteria inhibit the growth of putrefactive bacteria in the colon and promote the absorption of Ca^{2+} and PO^{4-} ions as well B vitamin synthesis. These bacteria can also stimulate the immune system. In this regard, the consumption of yacon root leads to an increase in short-chain fatty acids (SCFA) that protect against colon cancer (Delgado *et al.*, 2013).

Certain investigations have shown that yacon promotes several important aspects of health. An experiment using a diabetic rodent model showed that FOS consumption could improve insulin release and/or insulin-like activity. Some authors state that the saccharides present in yacon, particularly β-(2→1) fructooligosaccharides, could modulate the metabolic syndrome that occurs in type 2 diabetes and dyslipidemia, which are considered to be risk factors for atherosclerosis (Delgado *et al.*, 2013).

Low pH and the production of SCFA due to the consumption of prebiotics result in hypertrophy of the mucosal cells, enlargement of the intestinal surface and enhanced solubility of mineral ions. Yacon consumption for relatively short periods also resulted in increased intestinal absorption of minerals and bone mass, favoring the biomechanical properties of bone in rats. The enlargement of the cecal wall observed after yacon consumption appears to contribute to the increased mineral absorption in those animals. In fact, some studies show that daily consumption of a combination of short- and long-chain

inulin type fructans significantly increases calcium absorption and bone mineralization during pubertal growth. The effects of these dietary factors on calcium absorption appear to be modulated by genetic factors, including genetic polymorphism of a specific D-vitamin receptor. It has been observed that the consumption of inulin type fructans also reduces osteoporosis progression by increasing the bioavailability of calcium, with a significant increase in bone density and bone mineral mass (Delgado *et al.*, 2013).

2.16.1.3 Health benefits of yacon

Some of the most popular yacon health benefits include the control of blood sugar levels, control of cholesterol level, boosting immune system and helping in weight loss. But the most important is that yacon rich with carbs which is benefit to bring energy for daily activities. To get to know more brief information, below are several health benefits of yacon fruit.

- **Bioactivity and potential health benefits**

FOS is able to escape enzymatic digestion in the upper gastrointestinal tract, reaching the colon intact before undergoing microbial fermentation. FOS intake elicits a bifidogenic effect by selectively stimulating the proliferation of bifidobacteria, a group of beneficial bacteria naturally found in the human colon. Short chain fatty acids (SCFA), the endproducts of FOS fermentation by the intestinal microbiota, can also favor the growth of health-promoting bacteria such as *Bifidobacterium* spp. and *Lactobacillus* spp., while reducing or maintaining pathogenic populations (e.g., *Clostridium* spp. and *Escherichia coli*) at low levels. Thus, FOS are small soluble dietary fibers that exhibit prebiotic activity (Caetano *et al.*, 2016).

- **Control of type II diabetes**

Yacon contain inulin, which is a type of fructose found in the yacon tuber. Some researchers have claimed that it's useful in insulin independent diabetes, such as type II diabetes for regulating blood sugar levels. Research is still being continued and researchers are striving to document any importance of this plant in diabetes, which could be revolutionizing in the field of medicine as a therapeutic agent (Anon., 2009-2015).

- **Used as natural sweetener**

They can be used in the preparation of sugar free sweeteners for diabetic patients and those who are motivated to lose weight. Yacon sweetener can also be added in coffee and tea (Anon., 2009-2015).

- **Cancer prevention**

It is a potent anticancer agent for it inhibits the proliferation of mutant cells by initiating apoptosis (programmed cell death). It is found effective against skin, colon and blood cancer (Anon., 2009-2015).

- **Prevention of fatty liver**

Yacon is an important regulator of body fat and prevent excessive cholesterol accumulation, thus protecting liver function by assisting it in cholesterol metabolism. It helps in metabolic processes of body (Anon., 2009-2015).

- **Reduced triglycerides**

Yacon contain fructooligosaccharide that lowers bad cholesterol (triglycerides and low density lipoprotein). Low level of triglyceride is associated with reduce risk of heart attacks and stroke (Anon., 2009-2015).

- **Prebiotic effects**

It is associated with correction of digestion by increasing intestinal flora and preventing colitis (Anon., 2009-2015).

- **Cure for constipation**

It is a used to increase intestinal motility thus reducing constipation (Anon., 2009-2015).

- **Antioxidant activity**

Chemical analysis of yacon has shown antioxidant activity which prevents the body from inflammatory and chronic diseases. This is one of the chief yacon root benefits. These antioxidants were extracted by methanol from the yacon plant. Caffeic acid, ferulic acid and chlorogenic are the anti-oxidants found in the leaves of yacon (Anon., 2009-2015).

- **Anti-fungal**

Yacon leaves have reported important anti-fungal effects. The yacon leaf may be used in the treatment of fungal diseases like athlete's foot (Anon., 2009-2015).

- **Manage blood pressure**

Consuming yacon is also good to manage the blood pressure. Therefore, it is good for people with symptoms of hypertension. By keeping the blood pressure, it can also help to avoid various diseases related to hypertension such as heart attack. One of the killer disease in the world (Anon., 2017).

- **Control cholesterol**

Another health benefit of yacon is to manage cholesterol level inside the blood. It can work to manage the HDL and LDL level inside the blood and help to avoid blood cod. Through a better blood circulation, it can produce a better health and body system. This is the same health benefits accedes that can control the cholesterol level too (Anon., 2017).

- **Avoid cardiovascular diseases**

Yacon is good to manage the cardiovascular health. Therefore, it can work to avoid cardiovascular diseases such as the early symptoms of stroke. By frequent consume of the fruit, the body will manage a better cardiovascular condition and can avoid a heart attack too. Furthermore, it can help to balance the blood cells and avoid any fat inside the blood (Anon., 2017).

- **Avoid hypertension**

The fruit is able to lower down the blood pressure. It can work to manage the blood pressure level. Therefore, it can bring a huge benefit for people with symptoms of hypertension. A proper portion of the fruit daily can help to stabilize the blood pressure. This is the same health benefits of sword beans that can avoid high blood pressure too (Anon., 2017).

- **Rich of fiber**

Yacon is rich of fiber therefore; it can help to ease the digestive system. It will manage intestine bowel movement to be optimized and work faster. By fasten digest, the body will absorb important nutrient and work to avoid fat formation (Anon., 2017).

2.16.1.4 Uses of yacon and its potential in Nepal

Yacon can be eaten raw or cooked and have traditionally been used in fruit salads, jams, puddings, and juices. Their peeled skin, once dried, can also be used to make nutritious organic tea. Farmers in Brazil and Japan produce a number of processed yacon products, such as air-dried tuber slices unrefined yacon syrup that has a consistency of honey and can be marketed as a dietetic sweetener or a juice without addition of sweeteners, synthetic colorants and preservatives, with only small additions of vitamin C. The yacon tuberous roots serve as a source of raw material for the production of sweet pastries, fermented vegetables and ethanol; they can be used as "chips" in dehydrated form. Another product is yacon juice treated with active carbon powder to obtain its clarification, decolorisation and deodorization, acetic acid fermentation of yacon juice with *Acetobacter pasteurianus* for production of improved yacon vinegar containing natural fructooligosaccharides. Yacon slices and stripes retain crunchiness during cooking and could be used in Asian stir-fried dishes (Manrique *et al.*, 2005).

Geographically, the climate and agricultural conditions of Nepal are quite similar to those of the Andes in South America. Unfortunately, due to their resemblance to a vegetable and the general lack of knowledge, yacons are not as popular as initially imagined. However commercial production of yacon began in Nepal. As for now, yacon is produced in around 40 districts of Nepal. However of late, farmers are getting upset with slow sales and have reportedly ended up using yacon as cattle fodder or in liquor (alcoholic) making (Khatiwada, 2018). In Nepal works on yacon and its possible utilization has been started Shrestha (2015) conducted a study on preparation and quality analyses of yacon ready to serve (RTS) and wine. The commercial production of yacon syrup also started in Nepal. Perhaps in the future, innovative farmers will adopt newer crops from Latin America to Nepal as well and commercial farming and processing will take its place slowly (Karki, 2013).

2.16.2 *Sarpagandha* (*Rauwolfia serpentina*)

Sarpagandha is a small erect glabrous shrub about 1 to 3 feet in height, bearing white or pinkish flowers. It grows fairly wild in the united provinces, also in Bihar and eastern and western ghats. It is called 'Sarpa-gandha' in Sanskrit and 'Chota chand' in Hindi. The roots, the leaves and the juice have been considered of medicinal importance from the very early times and have attracted the attention of the practitioners of the indigenous system of medicine. It has been used as an anthelmintic, as an antidote against snake bite and bites of other poisonous insects, in diarrhea, dysentery, cholera and also as an ecbolic. In recent years interest has been stimulated in this drug, because of its well-marked hypnotic and sedative properties. It forms the chief if not the only constituent of the various 'insanity cures' which are so widely advertised in the lay press. Its use in the treatment of high blood pressure is of a very recent origin and is the outcome of the pharmacological investigations carried out on this drug. This use may be said to be, still in an experimental stage and hence any record of careful clinical observations, would be valuable in assessing the true value of this drug in the treatment of hyperpicsia (Bhatia, 1942).

2.16.2.1 Plant description

- **Macroscopical**

Pieces from 4 to 10 cm. long and 0.5 to 1.5 cm. broad, cylindrical, rarely branched, rootlets usually absent, outer surface yellowish with longitudinal ridges, fracture short, smoothed trancersely cut surface showing a large yellow, radiate, dense xylem, occupying about three quarters of the diameter (Rajbhandary, 1995b).

- **Microscopical**

Powdered drug yellowish coloured; cork cells of two tyoes, larger and smaller cells in alternating bomds, radially arranged, abundance of starch, strarch grains mostly rounded except few irregular, 9 to 15 µm in diameter, some parenchyma presence of prismatic crystals of calcium oxalate, tracheids with numerous bordered pits and pitted thickening, xylem fibres few with thick wall (Rajbhandary, 1995b).

2.16.2.2 Plant taxonomy

Botanical Classification of *Sarpaganda*

Kingdom	Plantae
Sub Kingdom	Viridiplantae
Infra Kingdom	Sterptophya(land plants)
Super Divison	Embryophyta
Division	Tracheophyta (vascular plants)
Sub division	Spermatophytina (seed plants)
Class	Magnoliopsida
Super Order	Asteranae
Order	Gentianales
Family	Apocynaceae
Genus	*Rauwolfia*
Species	*serpentina*

Source: Singh (2016)

2.16.2.3 Chemical composition of *Sarpaganda*

The quantitative determination of phytochemical constituents of *Rauwolfia serpentina* was summarized in Table 2.11. High quantity of flavonoids, saponins and alkaloids were found on *Rauwolfia serpentina*.

Table 2.11 Phytochemical composition of *Rauwolfia serpentina* expressed as mg/100 g dry weight

Phytochemicals of *Rauwolfia serpentina*	Contents
Alkaloids	48
Flavonoids	1.72
Phenols	1.86
Tannins	0.51

Source: Harisaranraj *et al.* (2009)

The various phytochemical compounds or secondary metabolites present in *R. serpentina* include alkaloids, phenols, tannins and flavonoids (Kumari *et al.*, 2013).

- **Alkaloids**

Alkaloids are large group of organic molecules which contain a heterocyclic nitrogen ring. These are brought about by different organisms such as animals and microbes, but a particularly diverse array of alkaloids is produced by plants. Approximately 10% of plant species are believed to produce alkaloids as secondary metabolites, where they work predominantly in providing defence against herbivores and pathogens. Pure isolated alkaloids and their synthetic derivatives are used as medicinal agents for their analgesic, antispasmodic and bactericidal effects (Okwu and Okwu, 2004). The alkaloids obtained from the root extract acts directly on central nervous system and thereby reduces blood pressure as compared to other blood-pressure lowering agents. *R. serpentina* root is reported to contain 0.7 – 3.0% of total alkaloids and about 0.1% of the active principle reserpine which is an indole alkaloid, present in the root. Hence, root biomass production of this plant could be of economic importance. On the basis of the structure there are three types of alkaloids namely, weak basic indole alkaloids, alkaloids of intermediate basicity and strong anhydronium bases. The various alkaloids identified in *Rauwolfia* include ajmaline, ajmalimine, ajmalicine,

deserpidine, indobine, indobinine, reserpine, reserpiline, rescinnamine, rescinnamidine, serpentine, serpentinine and yohimbine etc (Srivastava *et al.*, 2006).

Amongst all, resperine is the principle alkaloid which shows large number of clinical applications. Along with resperine, yohimbine, serpentine, deserpidine, ajmalicine and ajmaline are used to treat hypertension and breast cancer (Klushnichenko *et al.*, 1995). Reserpine is a pure crystalline single alkaloid, derived from the roots of *Rauwolfia* and was first isolated in 1952. It is a relatively weak tertiary base occurring in the oleoresin fraction of the roots and is useful in the treatment of hypertension, cardiovascular diseases and neurological diseases. The antihypertensive properties of *Rauwolfia* roots are attributed to reserpine (3,4,5-trimethyl benzoic acid ester of reserpic acid, an indole derivative of 18-hydroxy yohimbine type). It is the most prominent of all alkaloids and used mainly as a natural tranquillizer. Reserpine is now being utilized as a tool in physiologic studies of body functions and in pharmacological studies (Pullaiah, 2002).

The antihypertensive actions of reserpine are due to its depressant action on central nervous system (CNS) and peripheral nervous system by binding to catecholamine storage vesicles present in the nerve cell. This prevents the normal storage of catecholamines and serotonin in decline of catecholamine. It interferes with the function of autonomic nervous system by depleting the transmitter substance from the adrenergic neurons and possibly by activating the central parasympathetic system. These substances are mostly involved in controlling heart rate, cardiac contraction and peripheral resistance. It also helps in sedation and lowering of blood pressure, especially in cases of hypertension exacerbated by stress and sympathetic nervous system activity. Reserpine causes the release of 5-hydroxytryptamine (5- HT) from all tissues in which it is normally stored and results in increase of urinary metabolites (Prusoff, 1961).

- **Phenols**

Phenols are the secondary plant metabolites widely distributed in the plant kingdom mainly herbs, shrubs, vegetables and trees. The presence of phenols is considered toxic for the growth and development of various pest and pathogens. Presence of high quantity of total polyphenolic compounds in *R. serpentina* shows significant antidiabetic and hypolipidemic properties. In medicine, it is used as an expectorant and emulsifying agent. The presence of

phenolic compounds indicates that this can be used as anti-microbial agent (Azmi and Qureshi, 2013).

- **Flavonoids**

These are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancerous activity. Flavonoids in intestinal tract also lower the risk of heart disease. As antioxidants, flavonoids provide anti-inflammatory activity used for the treatment of diseases in herbal medicine (Del-Rio *et al.*, 1997).

- **Tannins**

The oxidation inhibiting activity of tannins is due to the presence of gallic acid and diagallic acid. 75 Tannins have astringent properties, they hasten the healing of wounds and inflamed mucous membranes. Thus, explain the use of *R. serpentina* in treating many disorders by traditional medicine healers in South eastern India (Kumari *et al.*, 2013).

2.16.2.4 Medicinal use of *Sarpagandha* (*R. serpentina*)

Muller (2015) has described the following medicinal use of *Sarpagandha*:

- It is used for the treatment of high blood sugar. Hence called as universal medicine for lowering blood pressure.
- It cures insomnia, hysteria and hypertension. *Rauwolfia* is found beneficial in relieving hysteria. One gram power of root can be taken 3 times with milk every day.
- It is also useful for in the treatment of cataract.
- It also cures plague and fever.
- *Sarpagandha* is used for the treatment of Schizophrenia.
- It is used in different countries as a sedative and tranquilizer.
- *Rauwolfia serpentina* is believed to cure anxiety, psychosis and epilepsy.
- It is also quoted for the treatment of colic and cholera.
- The root is used for the difficult childbirth.

- It is used to stimulate uterine contraction.
- It is used in various part of the world for the treatment of snake (cobra), scorpion or reptile bite and stings of any poisonous insects.
- The mixture of this with ginger and black pepper regularizes menstruation.
- The extract is also used against dysentery since ages.
- It is used in the treatment of hypochondria, mental disorders and a certain form of insanity. It is also known as Medicine for the mentally upset people and majorly sold in Bihar and UP. This herb is very beneficial in relieving insanity. Patients should take blood pressure examination before taking this drug.
- *Rauwolfia serpentina* has also been in use in the treatment of intractable skin disorder such as psoriasis, excessive sweating and itching.
- It is also used against irregular heart action in old ages.
- *Sarpagandha* also cures toxic goitre.
- It is also useful some gynecological problems like frigidity and moliminia.
- It also balances Vata and pitta in the body.
- *Sarpagandha* is also used for rheumatism, edema and intestinal diseases.
- It is also used against constipation and dizziness.

2.16.3 *Pakhanbed* (*Bergenia ciliata*)

The literal meaning of word *Pakhanbed* is, one that breaks stones. There are many plants that are known by this name due to their diuretic and lithotriptic (dissolving or destroying stone in the bladder or kidneys) activities. (Prabhakar, 2014)

B. ciliata belong (haw.) Sternb belongs to the family Saxifragaceae which consist of 30 genera and 580 species. *B. ciliata* commonly known as hairy *Bergenia* is a perennial herb found between the height of 800–3000 m throughout the temperate Himalayas from Afghanistan to Southeast Tibet (Chauhan *et al.*, 2012b). In Bhutan it is found in Deothang, Phuntsoling, Mongar and Ha districts. In India it is reported from Lushai hills, West Bengal, Arunachal Pradesh, Meghalaya, Himalayas (Kumaon), Kyongnosla, Karponanag, Gangtok in

Sikkim, district Almora Uttarakhand (Grierson and Long, 1983; Hasan *et al.*, 2013). In Nepal it occurs in Makanwanpur district, Karepalanchwok district and Dolakha district. In Pakistan it is distributed in northern parts mainly FATA region of Khyber Pukhtunkhwa province, Poonch valley, Swat, Abbottabad, Galliyat and Chitral. It was long since this plant has been used as medicines for the treatment of different human ailments. In Himalaya region many rural communities use *B. ciliata* for the treatment of various diseases. For century's rhizome of *B. ciliata* has been used for curing pulmonary infections, leucorrhea, piles and for dissolving bladder and kidney stones (Ahmad *et al.*, 2018).

Different Ayurvedic treatise mentioned this plant and recommended its uses for treatment of urinary stones. Charak Samhita (210 BC-170 AD) mentioned this plant under the name *Pakhanbed* and recommended it for painful micturition, for curing abdominal tumour and for breaking up calculi. Sushruta Samhita (170 AD- 340 BC) and Ashtang Hridaya (341 AD – 434 AD) also mentions it for uric acid calculi (Prabhakar, 2014).

2.16.3.1 Plant description

Perennial herb with short, thick, fleshy and procumbent stems, Rootstock very stout, Leaves ovate or round and 5-15 cm long at flowering time, Upper and lower surfaces hairy, becoming almost hairless in age, Flowers white, pink or purple, 3.2 cm in diameter, forming a cymose panicle with flexible flowering stem, 10-25 cm long leafless and styles. Plant roots are Rhizome, solid, barrel shaped, cylindrical, 1.5-3 cm long and 1-2 cm in diameter with small roots, ridges, furrows and root scars distinct, transversely cut surface shows outer ring of brown coloured cork, short middle cortex, vascular bundles and large central pith, odour, aromatic, taste, astringent (Prabhakar, 2014).

2.16.3.2 Plant taxonomy

Kingdom: Plantae- Plants

Subkingdom: Tracheobionta-Vascular plants

Superdivison: Spermatophyta- Seed plants

Division: Magnoliphyta- Floweringp lants

Class: Magnoliopsida- Dicotyledons

Subclass: Rosidae

Order: Rosales

Family: Saxifragaceae- Saxifrage family

Genus: *Bergenia*

Species: *ciliata*

Source: Prabhakar (2014)

2.16.3.3 Chemical composition (phyto-chemistry)

Phytochemical screening of *B. ciliata* showed the presence of terpenoids, tannins, flavonoids, saponins, steroids (Uddin *et al.*, 2012). Presence of alkaloids, tannins, flavonoids, coumarins and glycosides in *B. ciliata* rhizome (González-Castejón and Rodriguez-Casado, 2011). Gyawali and Kim (2012) reported 48 volatile organic compounds in *B. ciliata*. These 48 compounds were divided into 11 categories which are phenol (19%), alcohol (19%), volatile organic compound (VOCs) (16%), terpenoids (14%), fatty acids (8%), sterol (5%), glycosides (5%), carboxylic acids (5%), flavonoids (3%), cinnamic acid (3%) and nitro compounds 3%. The Major classes of phytochemicals reported in *B. ciliata* are as follows:

- **Phenols**

Phenols are the most important constituents of *B. ciliata*. Different phenolic compounds like bergenin, tannic acid, gallic acid, catechin, [10]-3-*O*-galloylcatechin and [10]-3-*O*-galloylepicatechin are present in *B. ciliata* (Chauhan *et al.*, 2012b; Vahabi and Eatemadi,

2016). Bergenin, catechin, (−)-3-*O*-galloylcatechin and [10]-3-*O*-galloylepicatechin were isolated from rhizome of the plant (Keri and Patil, 2014).

- **Bergenin**

Bergenin, also known as cuscutin which is the most abundant and important compound found in family Saxifragaceae. Chemical formula of bergenin is $C_{14}H_{16}O_9 \cdot H_2O$. 346.3 g per mole is the molecular weight of bergenin (Chauhan *et al.*, 2012a). Gurav and Gurav (2014) reported that rhizome of *B. ciliata* contain 0.75% bergenin.

- **Gallic acid**

Part of *B. ciliata* which possess gallic acid is seed. It is a type of phenolics also known as 3, 4, 5-trihydroxybenzoic acid (Fiuza *et al.*, 2004). Due to the presence of gallic acid this plant shows antioxidant, antiviral and antifunfal activities. It is used to treat psoriasis in ointments. Gallic acid is inhibitor of weak carbonic anhydrase (Chauhan *et al.*, 2000).

- **Tannic acid**

Tannic acid is basically a form of tannin which is a polyphenol present in *B. ciliata*. Its chemical formula is $C_{76}H_{52}O_{46}$. Tannic acid is also known as tannimum, gallotannin, quercotannic acid, acidum, tannicum, digallic acid, oak bark tannin and quercitannic acid (Ahmad *et al.*, 2018).

- **Catechin**

Catechin is present in *B. ciliata* rhizome (Kumar and Tyagi, 2013; Pokhrel *et al.*, 2014). Its chemical formula is $C_{15}H_{14}O_6$. Catechin is flavon-3-ol which is a kind of phenol present in plants as secondary metabolite and associated with -epicatechin or (+)-catechin. Catechin is also recognized as Cyanidanol, Cianidol, Catechuic acid, Catechinic acid and d-Catechin. It is expected that encapsulation of catechin in cyclodextrins enhanced its taste to use it as an additive (Kielhorn and Thorngate Iii, 1999).

- **Sterol**

Important phytosterol β-sitosterol is present in *B. ciliata* roots and leaves. Chemical formula of β-sitosterol is $C_{29}H_{50}O$. β-sitosterol is a waxy powder of white color having a characteristic smell. It is hydrophobic in nature (Ahmad *et al.*, 2018).

- **Glycoside**

Glycoside present in *B. ciliata* is Arubtin. Its chemical formula is $C_{12}H_{16}O_7$. Arbutin also called as Arbutoside hydroquinone β-d-glucopyranoside is found in rhizome of *B. ciliata* (Yuldashev *et al.*, 1993).

- **Flavonoid**

(+)Afzelechin is a flavonoid present in rhizome of *B. ciliata*. Its chemical formula is $C_{15}H_{14}O_5$ and IUPAC name is (2R, 3R)-2-(4-hydroxyphenyl)-3, 4-dihydro-2H- chromene-3, 5, 7-triol. It is also found in *B. ligulata* rhizome. Afzelechin show α-glucosidase inhibitory activity. Other flavonoids present in *B. ciliata* rhizome are quercetin 3-o-β-D xylopyranoside and quercetin 3-o-α-l-arbinofuranoxide (Ahmad *et al.*, 2018).

- **Fatty acid**

Fatty acids present in *B. ciliata* are decanoic acid and nonanoic acid with chemical formula $C_{10}H_{20}O_2$ and $C_9H_{18}O_2$ respectively (Gyawali, 2011).

- **Terpene**

Terpene present in *B. ciliata* rhizome are limonene with chemical formula $C_{10}H_{16}$ and lianalool $C_{10}H_{18}O$ (Ahmad *et al.*, 2018).

- **Other phytochemicals**

2-Pentanone, 2,4-Dimethyl-3-pentanone, Hexanal, 2-Methyl-1-propanol, Acetic acid, Heptanol, 2-Ethyl hexanol, 3-Pentanol, 2-Pentanol, Octanol, Pentanol, Heptanal, 3-Methyl-4-hexen-2-one, 2-Nitropropane, Hexanol, 2.4-Hexadienal, 2,4-nonadienal, Pentanoic acid, Hexanoic acid, Hexalactone, Isobutyrophenone, 5,6-Dihydro-2-pyranone, Methyl nonanoate, Methyl cinnamate, β-phellandrene, [E]-4-Hepten-2-one are present in the oil extracted from *B. ciliata* plant (Gyawali, 2011).

2.16.3.5 Medicinal uses of *Pakhanbed*

Pakhanbed is used in Ayurveda and Unani system of medicine for treatment of many diseases especially for urinary stones. The plant root has cooling, laxative, analgesic, abortifacient (abortion causing) and aphrodisiac properties.

The roots are used in treatment of vesicular calculi, urinary discharges, excessive uterine haemorrhage, diseases of the bladder, dysentery, menorrhagia, splenic enlargement and heart diseases. Ayurveda mentions, the roots as bitter, acrid, post digestion pungent and cool in potency. It is tridoshnashak (balances Vata, Pitta and Kapha). The medicinal uses of *Pakhanbed* are (Prabhakar, 2014):

1. Teething troubles: The roots are rubbed down and given with honey to children when teething.

2. Ear pain: The leave juice is extracted in mortar and pestle. This is used as ear drops to cure earache.

3. Intestinal parasites roundworms: About 10 g of root paste or juice is taken orally by human adults with the molasses, twice a day for 3-4 days.

4. Cuts, boils, wounds and burns: Dried roots paste is applied externally on affected body parts.

5. Urinary disorders, stomach disorders and urogenital complaints: Decoction of fresh roots is taken orally for treating these conditions.

6. Constipation: Root paste is taken with lukewarm water.

7. Dysentery: Approximately 5-10 g root powder is taken with fresh water, two times a day.

8. Fever: The root powder tea is given to treat fever.

Part III

Materials and Methods

3.1 Materials

3.1.1 Raw materials

Yacon root was obtained from local market of Dhankuta district. Yacon syrup containing 66°Bx was obtained from Nepaley Industry, Kathmandu. Herbs [*Sarpaganda* (*R. serpetina*) and *Pakhanbed* (*B. ciliata*)], and sugar were purchased from local market of Dharan. Wine yeast (Lalvin dried wine yeast EC 1118, USA) was purchased from Kathmandu.

3.1.2 Chemical

All chemicals used were of analytical grade and obtained from the campus.

3.1.3 Glassware and equipment

All glassware and equipment used were obtained from the campus. Microprocessor UV-vis spectrophotometer (Labtronics Model LT – 291, India) was for measuring absorbance.

3.2 Experimental method

3.2.1. Preparation of yacon juice, pulp and syrup wines

3.2.1.1 Preparation of yacon pulp and yacon juice

Yacon was washed thoroughly with water, peeled by using stainless steel knife and sliced. The slices were immediately dipped into water containing 100 ppm sulphur dioxide to inhibit enzymatic browning. The sliced yacon were pulped using 3 g citric acid and 87 mg potassium metabisulphite (KMS) per kg of slices. The pulp was divide in to two lots. One lot of the pulp was used for pulp fermentation and the other was strained through a double-folded muslin cloth to obtain yacon juice.

3.2.1.2 Preparation of musts for fermentation

3.2.1.2.1 Yacon juice must

The weight of yacon juice used for fermentation was noted and its TSS was adjusted at 24°Bx by adding cane sugar. The pH was adjusted in the range of 3.8 ± 0.1 using 20% citric

acid solutions. Potassium metabisulphite (KMS) was added to the ameliorated must to maintain sulphur dioxide content at 100 ppm. The amount of KMS added during pulping was taken into consideration while adjusting the must SO_2 content.

3.2.1.2.2 Yacon pulp must

The weight of pulp was noted and its TSS, pH and SO_2 contents were adjusted as described for the preparation of yacon juice must.

3.2.1.2.3 Yacon syrup must

Yacon syrup (66°Bx) was diluted to single strength yacon juice (11°Bx) with potable water and the TSS was adjusted at 24°Bx using cane sugar. The pH and SO_2 were maintained at 3.8 ± 0.1 and 100 ppm respectively as described for yacon juice must (3.2.1.2.1).

3.2.1.3 Fermentation

The glass containers to be used for wine fermentation were cleaned with detergent, rinsed with water containing 1000 ppm SO_2 and finally rinsed with boiled water. The three musts (yacon juice, pulp and syrup) were filled in to three separate glass containers up to their 70% volumetric capacity and cotton plugged. Wine yeast was pitched at the rate of 0.3 g/L of the must after 6 hrs of must sulphitation. The containers were cotton plugged and allowed for alcoholic fermentation at room temperature (26 ± 2°C). The containers were shaken twice a day till two days of fermentation. Once the active fermentation was over (after 14 to 16 days of fermentation), the containers were air locked and the passive fermentation was allowed till the fermentation was completely stopped as evidenced by the cease of bubbling.

3.2.1.4 Racking, pasteurization and bottling of wine

After the completion of fermentation, the wine was racked and pasteurized. For pasteurization, the wine was put in a heating vessel and heated with continuous stirring up to 70°C using a gas stove after which the fire was turned off and left for 10 min. During heating and holding periods the vessel was covered with a plate containing cold water in order to prevent the loss of alcohol due to evaporation. Then wine was cooled to room temperature (26 ± 2°C) and filled in to pre-cleaned and KMS treated wine bottles. The wines were analyzed for their chemical and sensorial characteristics and the best wine was selected.

3.2.2 Preparation of herbal wines

The yacon juice, which resulted the best wine among the three musts (yacon juice, pulp and syrup), was used for adding herbs (*Sarpaganda* and *Pakhanbed*) to prepare herbal wine. Yacon juice must was prepared as described in 3.2.1.2.1 and it was divided into three equal lots each of 4 L volume. *Sarpabanda* and *Pakhanbed* were ground to powder and were added into the first and second lots respectively at the rate of 10 g/L of the must. The third lot (without herb addition) was taken as control. Fermentation, racking, pasteurization and bottling of the wines were carried out as described in 3.2.1.3 and 3.2.14. The wines were subjected for chemical and organoleptic analyses. For sensory evaluation, herbs incorporated yacon juice wines were diluted with equal volume of control wine (without herb) and were subjected for sensory evaluation. The effects of herbs addition on the chemical and sensory characteristic of yacon juice wine were found out.

3.3 Analytical procedures

3.3.1 Determination of total acidity, fixed acidity and volatile acidity

The total- , fixed- and volatile acidity were determined as per Kirk and Sawyor (1991). Total and fixed acidities were expressed in % (m/v) as lactic acid, while volatile acidity was expressed in % (m/v) as acetic acid.

3.3.2 Determination of TSS and pH

TSS was determined using hand refractometer (Hanna Instrument, Portugal) and the results were expressed as °Bx. The pH was measured by using digital pH meter (Hanna Instrument, Portugal).

3.3.3 Determination of alcohol content

100 mL of the wine was neutralized with 0.1N NaOH and distilled as described by Kirk and Sawyor (1991). The alcohol content in the distillate was determined by spectrophotometric method as per Zoecklein *et al.* (1997) using UV-vis spectrophotometer.

3.3.4 Determination of total esters

Total esters content was determined by titrimetric method as per Kirk and Sawyor (1991). Briefly, 100 ml of the distillate was neutralized with 0.1 M NaOH and 10 ml of 0.1 M NaOH

solution was added to it. It was refluxed for 1 h using glass beads, cooled and titrated with 0.05 M H_2SO_4 solution. Similarly, a blank was also run using 100 ml distilled water instead of the distillate. Total esters content was calculated as follows:

Total esters (g ethyl acetate/100 L alc = 880 x V/S

Where, V = Blank titre – Sample titre, ml

S = Alcohol content in the distillate, % (v/v)

3.3.5 Determination of total aldehydes

Total aldehydes content was determined by titrimetric method as per Kirk and Sawyor (1991) and the results were expressed as gram acetaldehyde per 100 L of alcohol.

3.3.6 Determination of antioxidant activity

The antioxidant activity of wine was determined by DPPH method as per (Sing *et al.*, 2008). Briefly, wine sample was filtered through Whatman No. 41 filter paper. One ml of the filtered wine was diluted to 10 ml with distilled water. One ml of the diluted wine was taken in a test tube and 4 ml of 0.004% methanolic solution of DPPH was added. Then the test tube was incubated at room temperature (28°C) for 30 min in the dark and absorbance was measured at 517 nm using a UV-vis spectrophotometer. Similarly, blank was also run using methanol instead of the sample. The DPPH scavenging activity was calculated as follows:

DPPH scavenging activity (%) = (Blank absorbance – Sample absorbance) ×100/Blank absorbance

3.3.7 Determination of total phenolic (TP)

Total phenolic content was determined as per Sadasivam and Manickam (1996). Briefly, 1 ml of the filtered (Whatman 41 filter paper) wine was diluted to 10 ml with distilled water. One ml of the diluted wine was pipetted into a test tube and 2 ml of distilled water and 0.5 ml of Folin-ciocalteau reagents were added. After 3 min, 2 ml of sodium carbonate solution (20%) was added, mixed thoroughly and incubated at room temperature for 1h after which the absorbance was measured at 650 nm against a reagent blank. Total phenolic content in the wine was calculated from the standard curve prepared using different concentrations of gallic acid and the result was expressed as mg gallic acid equivalent (GAE)/100 ml wine.

3.3.8 Determination of fusel oil (Higher alcohol)

Higher alcohol was determined by spectrophotometric method as per AOAC (2005). Briefly, 1 g of the fusel oil standard (4 volumes isoamyl alcohol mixed with 1 volume of isobutyl alcohol) was diluted to 1 L with water. Finally, working standard solutions were prepared by pipetting 0, 5 10, 25 and 35 ml of fusel oil standard solution in to 100 ml volumetric flasks containing 7 ml of 95% neutral ethanol and diluting to volume with distilled water.

Distillate (1 ml) was pipetted in a test tube and diluted to 2 ml with distilled. One milliliter of DMAB (p-Dimethylaminobenzaldehyde) solution (1 g DMAB dissolved in a mixture of 5 ml H_2SO_4 and 90 ml distilled water and volume made up to 100 ml with distilled water) was added to the test tube, shaken and placed in ice bath for 3 min. With the tube still in ice bath, 10 ml of chilled H_2SO_4 was added into the tube, shaken and replaced in ice bath for 3 min. Then the tube was placed in a boiling water bath for 20 min and replaced in ice bath for 5 min. The tube was shaken and brought to room temperature. Similar procedure was followed for fusel oil working standard solutions. The transmittance (% T) of both the test sample and working standard solution were read at 540 nm against reagent blank as reference. The concentration of the fusel oil was found out from the fusel oil standard curve prepared by plotting gram fusel oil on linear scale as abscissa against %T as ordinate on log scale of semi log paper. The results were expressed as mg fusel oil/100 ml wine.

3.3.9 Determination of methanol content

Methanol content was determined by chromotropic acid colorimetric method as per AOAC (2005). Briefly, 2 ml of $KMnO_4$ solution (3 g $KMnO_4$ dissolved in a mixture of 15 ml H_3PO_3 and 85 ml distilled water) was pipetted into a 50 ml volumetric flask, chilled in ice bath. 1 ml of the distillate sample was added to the flask and stand for 30 min in ice bath. The excess of $KMnO_4$ solution was decolorized with 2% sodium sulphite solution and 1 ml of chromotropic acid solution (5% aqueous solution) was added. Then 15 ml of conc H_2SO_4 was slowly added with swirling and placed in hot water bath maintained at 70°C for 15 min and cooled. The volume was made up to 50 ml, and the absorbance was read at 575 nm against a reagent blank containing 5.5% ethanol treated similarly. Standard methanol solution (0.025% by volume in 5.5% ethanol) was also treated simultaneously in the same manner, and the absorbance recorded. Methanol content in the wine was calculated as follows:

Methanol content (%, v/v) = Sample absorbance × 0.025/ Standard absorbance

3.3.10 Determination of total reducing sugars

The wine samples were clarified using 45% neutral lead acetate solution and 22% potassium oxalate solution as described in (Ranganna,1986). Total reducing sugars was determined by Nelson-Somogyi method as per Sadasivam and Manickam (1996) and the results were expressed as mg glucose/100 ml wine.

3.3.11 Determination of glucose

Glucose content was determined by glucose oxidase peroxidase method as per Sadasivam and Manickam (1996). Briefly, 1 ml of the clarified wine solution (from total reducing sugar determination) was pipetted into a test tube containing 1 ml of distilled water and 1 ml of glucose oxidase peroxidase reagent was added. The content was mixed, incubated at room temperature (28°C) for 30 min and the absorbance recorded at 540 nm against blank containing distilled water treated similarly. The glucose content in the wine was calculated from the glucose standard curve.

3.3.11 Determination of fructose content

Fructose content was determined as per Sadasivam and Manickam (1996). Briefly, 2 ml of clarified wine solution (from total sugar determine), 1 ml of resorcinol (1 g of resorcinol and 0.25 g thiourea dissolved 100 ml glacial acetic acid) and 7 ml dil HCl (1 part HCl +5 parts water) were taken in a test tube, mixed and placed in a water bath maintained at 80°C for 10 min. The tube was cooled to room temperature and absorbance taken at 520 nm within 30 min against a blank consisting of distilled water instead of the sample. Fructose content was calculated using standard fructose curve and the result was expressed as mg fructose/100 ml wine.

3.3.12 Sensory evaluation

Sensory evaluation of the wines was carried out by using 5-points hedonic scale (1 = Poor, 2 = Fair, 3 = Satisfactory, 4 = Good, and 5 = Excellent) as per Ranganna (1986) with slight modification. Semi-trained panelists were requested to evaluate the samples in terms of taste, color, smell, flavor, mouthfeel and overall acceptability.

3.4 Data analysis

The experiment was performed using CRD with three replication. The data were analyzed using IBM SPSS Statistics, 20 and the treatment means were compared by Tukey test at 5% level of significance.

Part IV

Results and discussion

Chemical and volatile constituents of wines made from yacon juice, pulp and concentrate were analyzed and their sensory qualities were compared. Effects of *Pakhanbed* (*R. serpentina*) and *Sarpaganda* (*B. ciliata*) power addition on the chemical and sensory characteristics of wines made from yacon juice were studied.

4.1 Chemical characteristics of yacon juice, pulp and syrup wines

The chemical characteristics of wines made from yacon juice, pulp and syrup were analyzed and the results are shown in Table 4.1.

Table 4.1 Chemical characteristics of wines made from yacon juice, pulp and syrup

Characteristics	Wine samples*		
	Yacon juice	Yacon pulp	Yacon syrup
Total acidity as lactic acid (%, m/v)	0.59^{a} (0.02)	0.69^{b} (0.03)	0.86^{c} (0.03)
Fixed acidity as lactic acid (%, m/v)	0.56a (0.01)	0.59a (0.01)	0.72b (0.02)
Volatile acidity as acetic acid (%, m/v)	0.022^{a} (0.004)	0.058^{b} (0.008)	0.063^{b} (0.004)
pH	3.67^{ab} (0.06)	3.73^{b} (0.06)	3.53^{a} (0.06)
TSS (°Bx)	7.60^{a} (0.53)	7.67^{a} (0.58	8.33^{a} (0.58)
Antioxidant activity (DPPH inhibition, %) **	62.11^{a} (0.85)	42.88^{b} (4.34)	89.83^{c} (1.51)
Total phenolic (mg GAE/100 ml)	44.7^{a} (1.14)	40.9^{a} (1.16)	86.5^{b} (2.71)

*: values are the means of three determinations. Figures in the parentheses are standard deviations. Means having similar superscripts in a row are not significantly different ($p>0.05$).

**: 1 ml of wine was diluted to 10 ml with distilled water and used for the determination.

Wine made from yacon syrup had the highest total acidity (0.86%, m/v as lactic acid), while that of yacon juice had the lowest total acidity (0.59% m/v). Fixed acidity (FA) contents (% m/v as lactic acid) between yacon juice (0.56%) and pulp (0.59%) were statistically the same ($p>0.05$), while wine made from yacon juice concentrate had significantly the highest FA (0.72%) of all the wine samples.

The FA determined in yacon wines were greater than those reported by Egan *et al.* (1981) in various wine samples. Volatile acidity content was similar in wines made from yacon pulp and yacon juice syrup, while it was significantly lower in yacon juice wine compared to yacon pulp and yacon syrup wines. Similar results of volatile acids present in various wines were also reported by Egan *et al.* (1981) (0.03 – 0.2%, m/v). But the values of volatile acidity determined in this experiment were quite lower than those reported in white wine (0.27% m/v) and rose wine (0.3%, m/v) by Ancin *et al.* (1996). Vilanova *et al.* (2007) reported lower value of volatile acidity in red wine (0.3%, m/v).

The pH of yacon pulp wine (3.73) was significantly higher from that of yacon syrup wine (3.53), but it did not differ from that of yacon juice wine (3.67). The pH values of yacon wines found in this study were slightly higher than those reported in rose wine (3.0) and white wine (3.33) by Ancin *et al.* (1996).

No significant difference in TSS among the three wines were found. The antioxidant activity of wines differed significantly from each other, with the highest value being for yacon syrup wine (89.83% and lowest being for yacon pulp wine (42.88%). Total phenolic content (mg GAE/100 ml) was maximum in yacon syrup wine (86.5), but the values for yacon juice and yacon pulp wines were statistically similar. Analogous results of total phenolics was also reported by Vilanova *et al.* (2007) in three red wine samples (35.81 – 55.66 mg GAE/100 ml).

4.2 Volatile constituents of yacon juice, pulp and syrup wines.

The volatile contents viz., alcohol, total esters, total aldehydes, higher alcohols and methanol present in the yacon juice, pulp and syrup wines were determined and the results are shown in Table 4.2.

Table 4.2 Volatile constituents of yacon juice, pulp and syrup wines

Volatiles constituents	Wine samples*		
	Yacon juice	Yacon pulp	Yacon syrup
Alcohol (%, v/v)	12.5^{a} (0.24)	11.0^{b} (0.50)	10.67^{b} (0.76)
Total esters as ethyl acetate (g/100 L alc)	55.05^{a} (3.20)	46.36^{b} (1.60)	54.53^{a} (2.19)
Total aldehydes as acetaldehyde (g/100 L alc)	0.25^{ab} (0.03)	0.21^{a} (0.01)	0.29^{b} (0.01)
Higher alcohols (mg/100 ml wine)	17^{a} (0.78)	22^{b} (0.95)	19.3^{a} (0.66)
Methanol (%, v/v)	0.0014^{a} (0.00025)	0.0016^{a} (0.00015)	0.0017^{a} (0.00015)

*: values are the means of three determinations. Figures in the parentheses are the standard deviations. Means having similar superscripts in a row are not significantly different ($p>0.05$).

Alcohol content of yacon juice wine (12.5%, v/v) was significantly highest than those of yacon pulp and yacon syrup wines, but the values between the latter two wines did not differ significantly. The alcohol contents found in this study were similar o those reported by Egan *et al.* (1981) in different wines (7.5 – 14 g/100 ml wine). The alcohol contents found in this experiment were higher than those reported in three red wines (9.86 – 9.46%, v/v) by Vilanova *et al.* (2007). Total ester contents (g ethyl acetate/100 L alc) between yacon juice

wine (55.05) and yacon syrup wine (54.53) were similar, but it was significantly lower in yacon pulp wine (46.36) compared to the former two wines. Total ester content in yacon wines found in this study was in the range reported by (Clarke and Bakker, 2004) (25 – 300 mg/l of wine). Yacon syrup wine had significantly higher total aldehyde content than that of yacon pulp wine, but the value for yacon juice wine was statistically similar to those of yacon pulp and syurp wines. Total aldehydes content in yacon wines was lower than that reported by Briggs *et al.* (2004) (10 – 20 mg/L wine). The principal causes of high acetaldehyde concentrations in wine are the use of poor quality pitching yeast, excessive must oxygenation, unduly high fermentation temperature and excessive pitching rates (Briggs *et al.*, 2004).

Higher alcohol content was maximum in yacon pulp wine (22%, v/v) compared to yacon juice and syrup wines, but the values between the latter two wines were statistically similar. The higher alcohols content in wine should be 80-540 mg/L and the concentration of higher alcohols below 300 mg/L contributes the desirable aroma of wine, whereas these components are seen as a negative factor in creating the aroma when their level exceeds 400 mg/L (Usansa, 2003). Conditions which favor increased yeast growth, such as excessive aeration or oxygenation, promote higher alcohol formation, but this can be ameliorated by the application of a top pressure during fermentation (Baxter and Hughes, 2001). Methanol content ranged from 0.0014 to 0.0017% (v/v) in the three wines; however, the values were not significantly different. The methanol contents in wines were with the range (0.1 – 0.2 g/L) reported by (Jackson, 2014).

4.3 Sensory quality of wines made from yacon juice, pulp and syrup.

The sensory quality of wines were evaluated in terms of their color, taste, flavor, mouthfeel and overall acceptance using 5-points hedonic scale and the results are shown in Fig. 4.1.

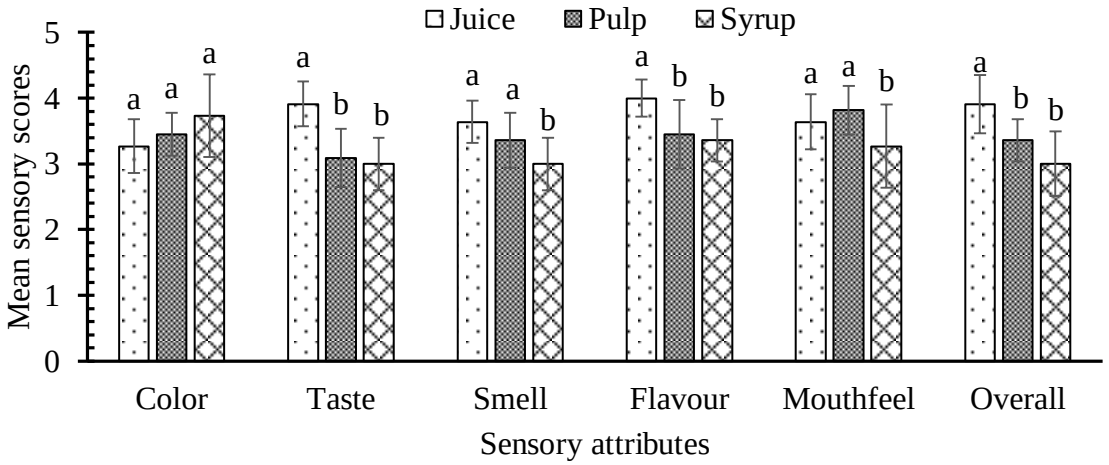

Fig.4.1 Mean sensory scores for juice, pulp and syrup wine

*: Values are the means of 10 panelists. Error bars indicate 95% CI. Similar letter for any quality attribute are not significantly different ($p>0.05$).

The mean color scores for yacon juice, pulp and syrup wines were 3.27, 3.45, and 3.73 respectively. Statistical analysis revealed that the scores were not significantly different ($p>0.05$). The average taste scores were 3.91, 3.09 and 3.0 for wine made from yacon juice, pulp and syrup respectively. Statistically, yacon juice wine had significantly the highest taste score compared to yacon pulp and yacon syrup wines, whereas the taste scores for the latter two wines were similar. In terms of the taste preference, yacon juice wine was rated as "good", while yacon pulp and yacon syrup wines were rate as "satisfactory" by the sensory panelists.

The mean smell preference scores for yacon juice, pulp and syrup wines were 3.64, 3.36 and 3.0 respectively. The mean flavor scores were 4.00, 3.45 and 3.36 for yacon juice, pulp and syrup wines respectively. There is no significant difference.

The average mouthfeel scores were 3.64, 3.82 and 3.27 for yacon juice, pulp and syrup wines respectively. The mouthfeel preference scores between yacon juice and pulp wines were statistically similar. Overall acceptability scores for yaon juice, pulp and syrup wines were 3.91, 3.36 and 3.00 for wines made from yacon juice, pulp and syrup respectively.

Statistical analysis showed that yacon juice wine had the highest overall acceptability score (3.91 = rated as nearly "good") of all the wine samples.

Both the volatile and non-volatile constituents' analysis revealed that yacon syrup wine had the highest antioxidant activity and total phenolics contents, but it had significantly lower alcohol content. Moreover, total esters, total aldehydes and higher alcohol contents between yacon juice and yacon pulp wines were similar. Based on the sensory evaluation of wines, most of the sensory attributes were significantly higher for wind made from yacon juice compared to yacon pulp and syrup. Thereforje, yacon juice was selected as a raw material for herbal wine preparation using *Pakhanbed* and *Sarpaganda*.

4.4 Effects of *Pakhanbed* and *Sarpaganda* addition of the quality of yacon juice wine

Sarpaganda and *Pakhanbed* were separately incorporate at the rate of 10 g/L of the yacon juice must and fermented. After fermentation, both control and herbs added wines were analyzed for their chemical and sensory characteristics.

4.4.1 Effect of herbs addition on the chemical constituents of yacon juice wine

The chemical characteristics in terms of total acidity, fixed acidity, volatile acidity, pH, TSS, total reducing sugar, glucose, fructose, and total phenolic content (TPC) were analyzed and the results are shown in Table 4.3.

The total acidity contents for control and *Pakhanbed* incorporated wines were statistically similar, whereas that of *Sarpaganda* added wine had significantly the highest total acidity (0.71% m/v as lactic acid) of all the wines. Similar trend was also found in the case of fixed acidity contents of wines, with the highest value being for *Sarpaganda* incorporated wine (0.69% m/v as lactic acid). However, the volatile acidity contents among three wines were statistically not different, with the values being in the range of 0.015 – 0.02% m/v as acetic acid. The pH values of wines ranged from 3.63 to 3.77, but the values were statistically not different. Statistical analysis showed that herbs incorporation had no significant effect ($p>0.05$) on the TSS of yacon juice wine and the values ranged from 7.73 to 7.93°Bx.

The total reducing sugar contents (mg glucose/ 100 ml) were similar in *Pakhanbed* added wine (243.9) and *Sarpaganda* added wine, while that of control had significantly the lowest (115.7) of all the wines. Glucose content was minimum in control wine (3.7%, m/v) while *Pakhanbed* and *Sarpaganda* added yacon juice wines had more than 2-fold glucose content

compared to control. Fructose contents among the three wines did not differ significantly, and the values ranged from 50.1 to 60.2%, m/v. Total phenolics contents (mg GAE/100 ml) between control wine (42.7) and *Sarpaganda* added wine (47.1) were similar, but that of *Pakhanbed* added wine was significantly higher (103.5) compared to former two wines.

Table 4.3 Chemical characteristics of *Pakhanbed* and *Sarpaganda* incorporated yacon juice wine.

Characteristics	Wine samples*		
	Control	*Pakhanbed*	*Sarpaganda*
Total acidity as lactic acid (%, m/v)	0.58^{a} (0.005)	0.57^{a} (0.005)	0.71^{b} (0.004)
Fixed acidity as lactic acid (%, m/v)	0.55^{a} (0.003)	0.55^{a} (0.004)	0.69^{b} (0.01)
Volatile acidity as acetic acid (%, m/v)	0.02^{a} (0.002)	0.015^{a} (0.003)	0.015^{a} (0.003)
pH	3.67^{a} (0.06)	3.77^{a} (0.06)	3.63^{a} (0.06)
TSS (°Bx)	7.93^{a} (0.12)	7.73^{a} (0.17)	7.87^{a} (0.12)
Total reducing sugars as glucose (mg/100 ml)	115.7^{a} (14.5)	243.9^{b} (5.5)	251.4^{b} (14.8)
Glucose (mg/100 ml)	3.7^{a} (0.4)	7.8^{b} (0.8)	8.1^{b} (0.6)
Fructose (mg/100 ml)	60.2^{a} (3.8)	50.7^{a} (8.0)	50.1^{a} (6.4)
Total phenolic (mg GAE/100 ml)	42.7a (3.3)	103.5b (1.4)	47.1a (1.8)

*: values are the means of three determinations. Figures in the parentheses are standard deviations. Means having similar superscripts in a row are not significantly different ($p>0.05$).

Pakhanbed and *Sarpaganda* was separately added to the yacon juice must at the rate of 10 g/L prior to fermentation. Wine made from yacon juice only was taken as control.

Alcohol contents among the three wine samples were statistically similar and the values ranged from 12.5 to 13.01% (v/v), indicating that addition of herbs had no significant effect on the alcohol content of yacon juice wine. Similar result was also found in the case of total ester contents of the wines, the values being in the range of 54.72 – 63.39 g ethyl acetate/100 L alc. Addition of herbs did not have significant effect on the total aldehydes

contents of wines, and the contents ranged from 0.23 to 0.25 g acetaldehyde/100 L alc. Both higher alcohols and methanol contents of wine samples did not differ from each other, in which higher alcohols contents ranged from 17.6 to 19.4 mg/100 ml wine and methanol contents ranged from 0.0011 to 0.0015%, v/v.

4.4.2 Effects of herbs addition on the volatile constituents of yacon juice wine

Volatile constituents, viz., alcohol, total esters, total aldehydes, higher alcohols, and methanol contents of both the control herbs (*Sarpaganda* and *Pakhanbed*) added yacon juice wines were analyzed and the results are shown in Table 4.4.

Table 4.4 Volatile constituents of *Pakhanbed* and *Sarpaganda* added yacon juice wine.

Volatiles	Wine samples*		
	Control	*Pakhanbed*	*Sarpaganda*
Alcohol (%, v/v)	13.01[a] (0.50)	12.5[a] (0.31)	12.67[a] (0.45)
Total esters as ethyl acetate (g/100 L alc)	54.72[a] (4.24)	63.39[a] (2.86)	58.14[a] (3.51)
Total aldehydes as acetaldehyde (g/100 L alc)	0.25[a] (0.04)	0.27[a] (0.02)	0.23[a] (0.01)
Higher alcohols (mg/100 ml wine)	17.6[a] (2.1)	18.1[a] (2.1)	19.4[a] (1.0)
Methanol (%, v/v)	0.0011[a] (0.000)	0.0013[a] (0.000)	0.0015a (0.000)

*: values are the means of three determinations. Figures in the parentheses are standard deviations. Means having similar superscripts in a row are not significantly different ($p>0.05$). *Pakhanbed* and *Sarpaganda* powder were separately added to the yacon juice must before fermentation.

4.4.3 Effect of herbs addition on the antioxidant activity of yacon juice wine.

The antioxidant activities of control and herbs added wines were determined and a graph of DPPH free radical scavenging activity against incubation time was plotted to study the trend of antioxidant activity with time (Fig. 4.2.)

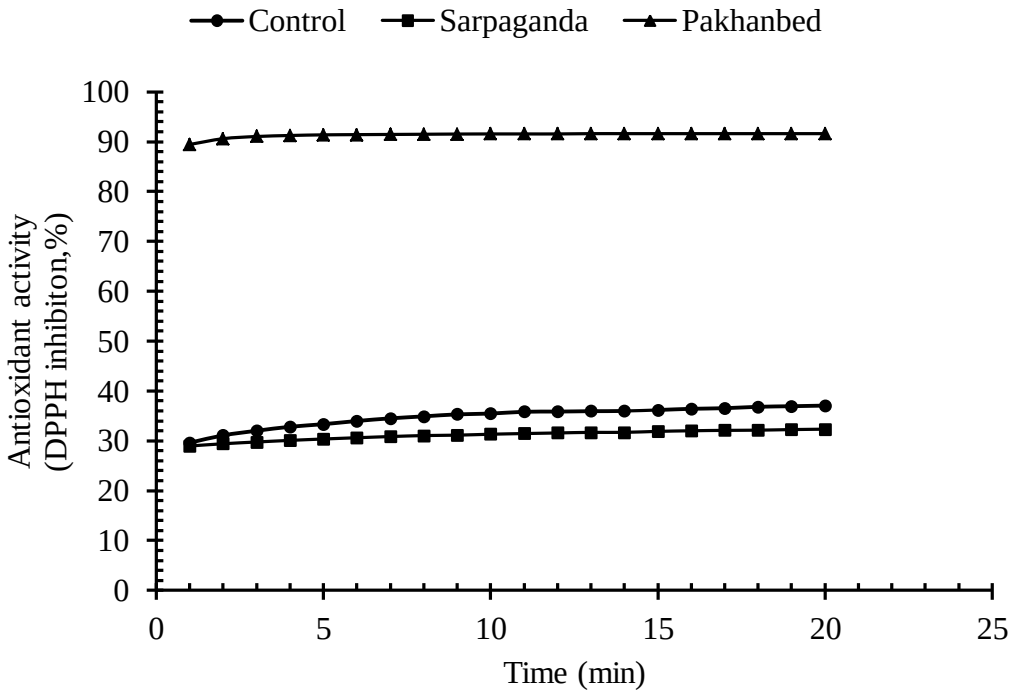

Fig. 4.2 Effect of herbs addition on the antioxidant activity of yacon juice wine.

*: values are the means of triplicate determinations. 1 ml of wines were diluted to 10 ml with distilled water and used for the determination.

From Fig 4.2 it was found that antioxidant acidity increased with increase in incubation time. The increment was fastned in *Pakhanbed* incorporated wine, where an antioxidant activity of 91% was reached within 3 min of incubation and remained almost constant afterwards. The Statistical analysis showed that addition of herbs had a significant effect on the antioxidant activity of yacon juice wine. Antioxidant activities of control and *Sarpaganda* added yacon juice wines were 37.06 and 32.33% respectively over 20 min of incubation, but the values were statistically not different.

4.4.4 Effects of herbs addition on the sensory quality of yacon juice wines

Preliminary study showed that both he herbal added wines were more bitter in taste, and hence; the wines were diluted with the equal volume of control wine and subjected for sensory evaluation. The sensory quality of herbs added yacon juice wines were evaluated in terms of color, taste, smell, flavor, mouthfeel and overall acceptability using 5-points hedonic scale and the results are shown in Fig. 4.3.

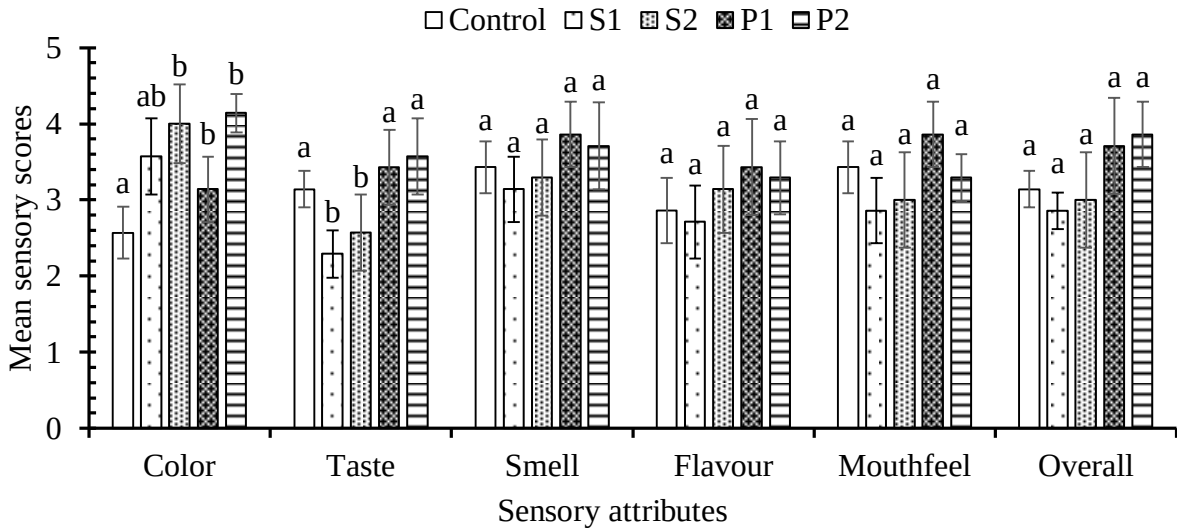

Fig. 4.3 Effects of *Sarpagandha* and *Pakhanbed* addition on the sensorial quality of yacon wine

*Values are the means of ten sensory panelists. Error bars indicate the 95% CI. Similar letters on the bars for any quality attribute indicate insignificant different ($p>0.05$)

Control: yacon juice wine made without adding herbs

S_1: yacon juice fermented by adding 0.5% (m/v) of *Sarpaganda*

S_2: yacon juice fermented by adding 1% (m/v) of *Sarpaganda*

P_1: yacon juice fermented by adding 0.5% (m/v) of *Pakhanded*

P_2: yacon juice fermented by adding 1% (m/v) of *Sarpaganda*

The mean color scores of control, 0.5% (m/v) of *Sarpaganda*, 1% (m/v) *Sarpaganda*, 0.5 % (m/v) *Pakhanbed* and 1% (m/v) *Sarpaganda* added yacon juice wines were 2.57, 3.57, 4.00, 3.14, and 4.14 respectively. Statistical analysis showed that yacon juice fermented by

incorporating with both herbs (*Sarpaganda* and *Pakhanbed*) at both concentration levels had similarity in terms of color preference of the wines. Both *Sarpaganda* and *Pakhanbed* when added at the rate of 1% (m/v) of the yacon juice must significantly improved the color preference of wine compared to control. The mean taste scores of control, S_1, S_2, P_1, and P_2 wine samples were 3.14, 2.29, 2.57, 3.43, and 3.57 respectively. It was observed that addition of *Sarpaganda* at both levels significantly impaired the taste preference of the yacon juice wine.

Addition of both herbs at either concentrations had no significant effect ($p>0.05$) on the mean smell scores of yacon juice wine, with the mean smell scores being in the range of 3.14 – 3.86. All wine samples were rated as "satisfactory" by the sensory panelists with respect to smell. Addition of herbs did not have significant effect on the flavor preference of wines, where mean flavor scores remained in the range of 2.71 – 3.43. Both mouthfeel and overall acceptability of yacon juice wines were not affected by the addition of *Sarpaganda* and *Pakhanbed* at both concentrations, where mean scores for mouthfeel and overall acceptability were in the range of 2.71 – 4.43 and 2.86 – 3.86 respectively.

Chemical analysis revealed that alcohol, total esters, total aldehydes, higher alcohols and methanol contents among the control, *Pakhanbed* and *Sarpaganda* added wines were not different. Other chemical characteristics so analyzed were also in the range of other wines reported in the literatures. Mean scores for most of the sensory quality attributes were higher for *Pakhanbed* added wine of all the wines evaluated. Furthermore, *Pakhanbed* added wine had surprisingly the highest antioxidant activity (91.62%) among all wines. Therefore, addition of *Pakhanbed* at the rate of 0.5% (m/v) yacon juice must could significantly enhance the medicinal value of wine without significantly affecting its chemical and sensory properties.

Part V

Conclusions and recommendations

Based on the results and discussion, the following conclusions were drawn from this study

5.1 Conclusions

1. Yacon juice wine was relatively better than those of yacon pulp and syrup wines in terms of their chemical and sensory characteristics.
2. Addition of *Pakhanbed* at the rate of 0.5% (m/v) of the yacon juice must significantly enhanced the antioxidant of the wine without scarifying its chemical and sensory attributes.
3. Addition of *Sarpagandha* made the wine more bitter than that of *Pakhanbed.*

5.2 Recommendations

1. Antidiabetic, antihypertensive and anti-stone properties of yacon-based herbal wines could be studied.
2. Locally available herbs can be used for the preparation of herbal wine.

Part VI

Summary

Different herb incorporated wines are in practice throughout the world. *R. serpentine* has been used as anthelmintic, an antidote against snake bite in diarrhea, dysentery and cholera. *B. ciliata* has been recommended for the treatment of urinary stones, painful micturition and for curing abdominal tumor. Therefore, this study was carried out aimed at exploring the possibility of incorporating *B. ciliata* and. *R. serpentina* in producing herbal wine, thus promoting commercial cultivation of these endangered Nepalese medicinal plants. Wines were prepared from yacon juice, yacon pulp and yacon syrup musts (24°Bx TSS, 3.8 ± 0.1 pH and 100 ppm SO_2) and subjected for chemical and sensory analyses to select the best wine. Yacon juice must was used for preparing herbal wine by adding *R. serpentina* and *B. ciliata*) separately at the rate of 1% (m/v) of the must.

Total and fixed acidities were higher in yacon syrup wine compared to yacon juice and pulp wines. No significant difference in TSS was found among the wines. Antioxidant activity was highest in yaon syrup wine (89.83%), while it was lowest in yacon pulp wine (42.44%). Total phenolics content was more than 2-fold in yacon syrup wine compared to yacon juice and pulp wines. Yacon juice wine had higher alcohol content (12.5%, v/v) than those of others. Total esters, total aldehydes and higher alcohol contents of the three wines ranged from 46.36 to 55.05 g ethyl acetate/100 L alc, 0.21 to 0.29 g acetaldehyde/100 L alc) and 17.0 to 22 mg/100 ml wine respectively. Methanol contents did differ among the wines. Yacon juice wine had better sensory quality of all the wines tested.

The pH, TSS, fructose and volatile acidity among control and herb added wines were not different. *Pakhanbed* significantly increased total phenolic (103.5 mg GAE/100 ml) compared to control and *Sarpaganda* added wines. Total reducing sugars was lowest in control compared to *Pakhanbed* and *Sarpaganda* added wines. Herbs addition did not affect alcohol, esters, aldehydes, fusel oil and methanol contents of yacon juice wine. Sensory evaluation indicated that *Pakhanbed* added yacon juice wine had higher sensory preference scores of all the wines evaluated. Therefore, addition of *Pakhanbed* at the rate of 5 g/L of yacon juice must could significantly enhance the medicinal value of wine without significantly affecting its chemical and sensory properties.

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APPENDICES

A1. Sensory evaluation score card by 5-points Hedonic rating test

Test the samples and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describe your feeling about the sample. Please give a reason for this attitude. Remember you are the only one who tell what you like. An honest expression of your personal feeling will help us in selecting the best sample.

Attributes	Samples				
	A	B	C	D	E
Color					
Taste					
Smell					
Flavor					
Mouthfeel					
Overall					

Score card

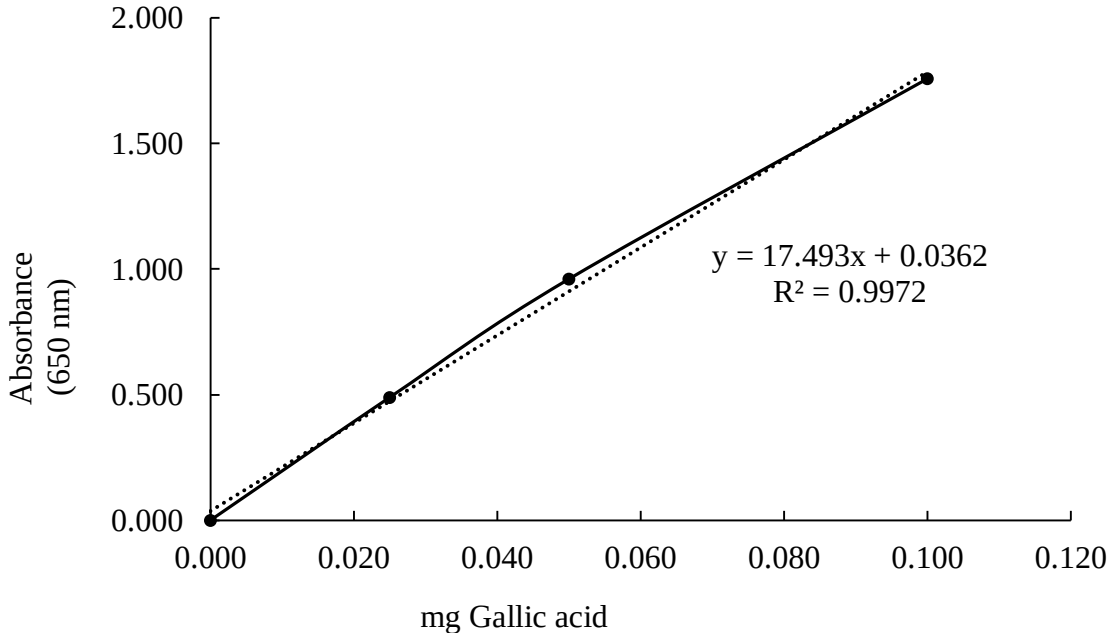
Score	**Feelings**
1	Poor
2	Fair
3	Satisfactory
4	Good
5	Excellent
6	

Comments (if any):……

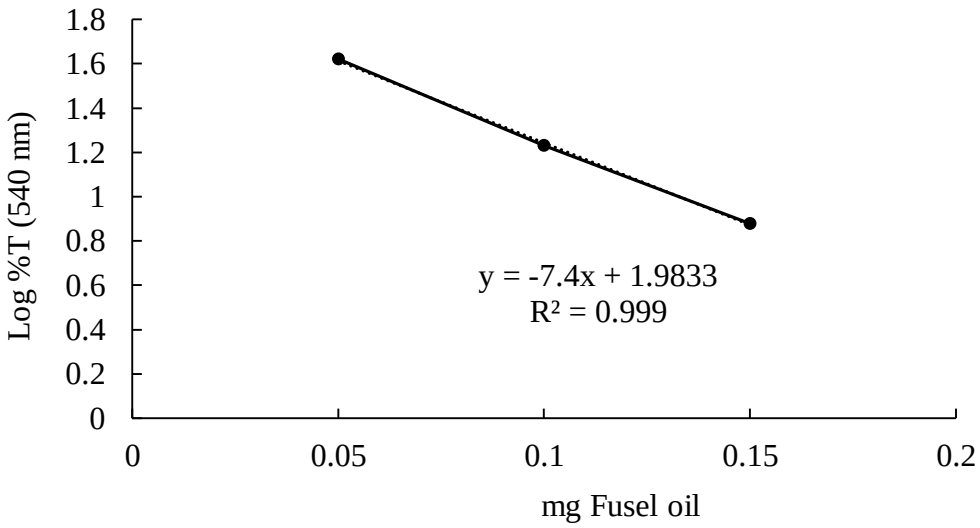
Name of the evaluator's:

Signature:…….. Date:……..

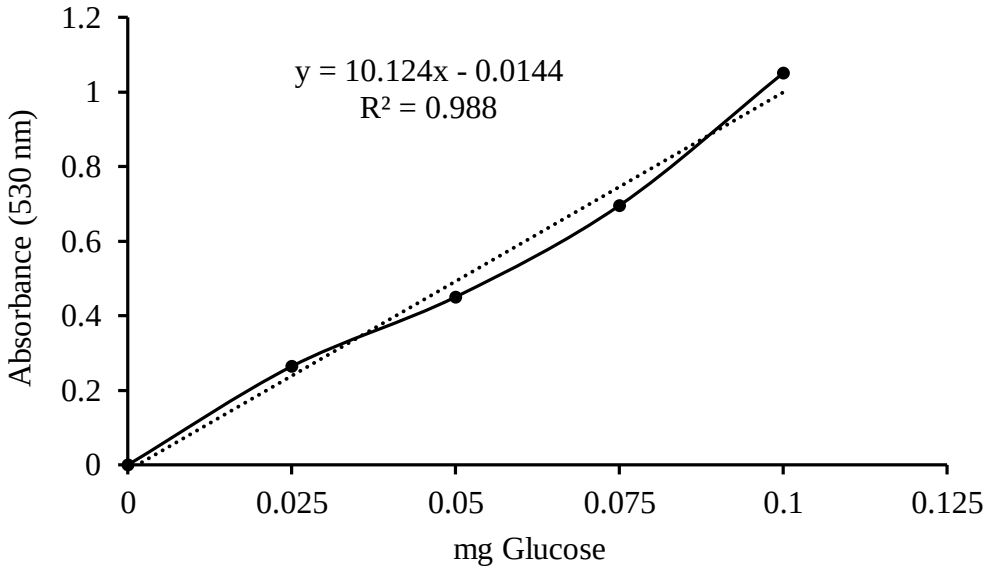
A2. Standard curve for total phenolic determination.



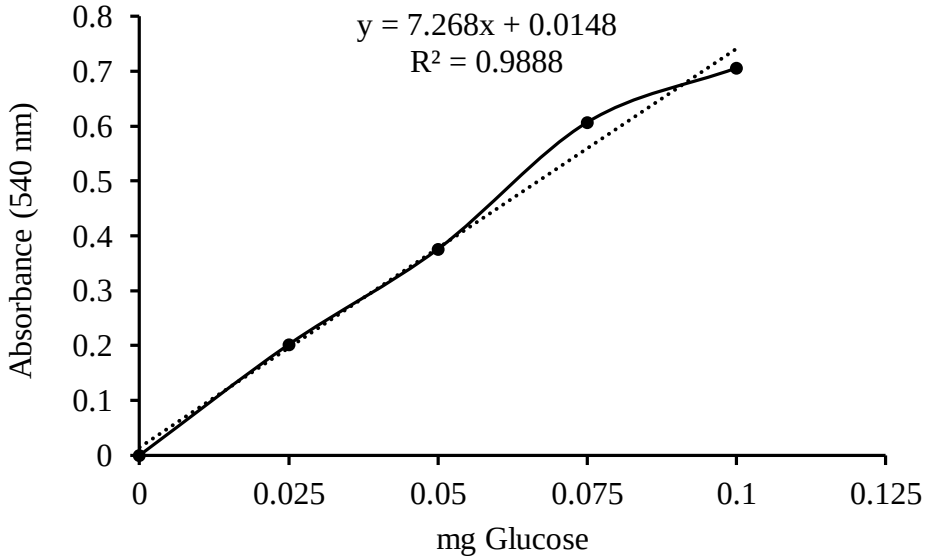
A3. Standard curve for higher alcohols determination



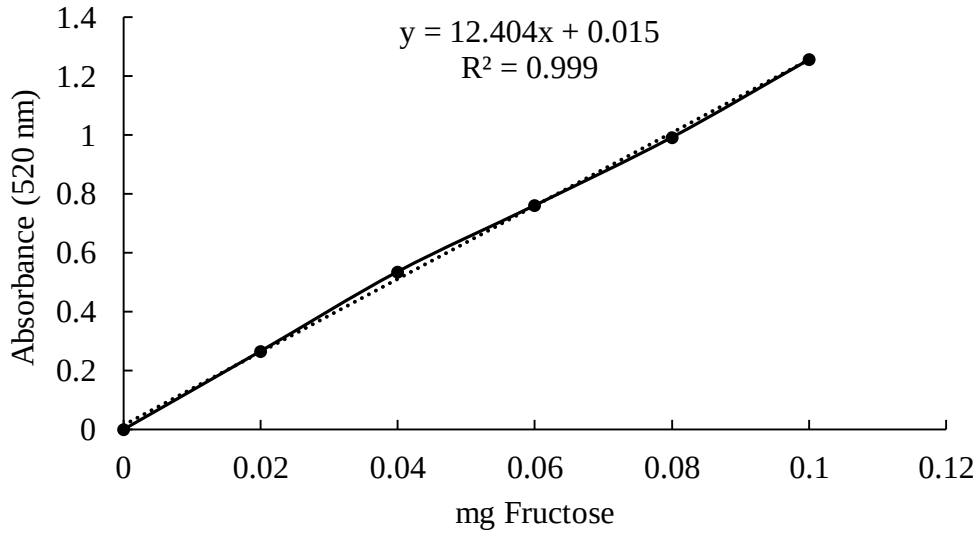
A4. Standard curve for total reducing sugars determination



A5. Standard curve for glucose determination



A6. Standard curve for fructose determination



A7. Standard curve for alcohol determination