EFFECT OF VARYING QUANTITIES OF *Tinospora sinensis* AND *Piper betle* EXTRACTS ON THE SENSORY AND PHYSICOCHEMICAL QUALITIES OF RUM

by

Anup Poudel Upadhyay

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2022

Effect of Varying Quantities of *Tinospora sinensis* and *Piper betle* Extracts on the Sensory and Physicochemical Qualities of Rum

A dissertation to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfilment of the requirement for the degree of B.Tech. in Food Technology

by

Anup Poudel Upadhyay

Department of Food Technology

Central Campus of Technology, Dharan

Institute of Science and Technology

Tribhuvan University, Nepal

2022

Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This ***dissertation*** **entitled** ***Effect of Varying Quantities of Tinospora sinensis and Piper betle Extracts on the Sensory and Physicochemical Qualities of Rum*** presented by **Anup Poudel Upadhyay** has been accepted as the partial fulfilment of the requirements for the **B.Tech. degree in Food Technology**.

Dissertation Committee

1. **Head of the Department** _____
   (Mr. Navin Gautam. Asst. Prof.)
2. **External Examiner** _____
   (Mr. Birendra Kumar Yadav, Asst. Prof)
3. **Supervisor** _____
   (Mr. Basanta K. Rai, Prof.)
4. **Internal Examiner** _____
   (Mrs. Babita Dahal Adhikari, Assoc. Prof.)

November, 2022

Acknowledgements

I would like to thank my supervisor, Prof. Mr. Basanta Kumar Rai whose, support, guidance, encouragement, recommendations and expertise were invaluable in formulating the research questions and methodology and pushing me to sharpen my thinking and bringing this work to a higher level.

I would like to express gratitude to Prof. Dr. Dil Kumar Limbu (Campus Chief, Central Campus of Technology) and Asst. Prof. Navin Gautam (Chairperson, Department of Food Technology, Central Campus of Technology) for providing me with all the facilities that I needed to successfully complete my dissertation.

I would like to acknowledge my senior Mr. Sanil Joshi, Aadarsha Paudel, Sanjog Kharel, Niraj Adhakari from whom I have received a great deal of support and assistance throughout the work. Special thanks to my friends; Tikaram Pahadi, Trilochan Pandey, Ranjan Shrestha, Roshan Bhattarai, Anjan Bhattarai and my beloved juniors; Mohit Khadka, Kshitiz Luitel, Samyog Sangroula, Hari Pokhrel, Milan Bolakhe, Prakash Sapkota, from Central Campus of Technology for their help and support during the completion of this work. I would also like to thank all those who have helped me directly or indirectly throughout the course of the work. I would also like to express my sincere thanks to my teachers, staffs of library and laboratory for their direct and indirect co-operation and suggestion.

Finally, my deep and sincere gratitude to my family for their continuous and unparalleled love, help and support. I am forever indebted to my parents for giving me the opportunities and experiences that have made me who I am.

Date of Submission: November, 2022

_____

(Anup Poudel Upadhyay)

Abstract

Utilization of herbs in rum provides antioxidant and effective flavor along with desirable color without caramelization. In the present study, extracts of two types of herbs viz., *gurjo* (*Tinospora sinensis*) stem and betel (*Piper betle*) leaf were used (separately) to prepare herbal rums. The said herbs were extracted in 40% alcohol (obtained by a separate pot-distillation of molasses wash). The amounts of herb extracts to be used in the experimental rums were determined through threshold test for appearance, taste, flavor and after-taste by sensory analysis (9-point hedonic scale) of trial rums containing (separately) gurjo stem - and betel leaf extracts in the range 0.1-0.4% and 0.6-2.4%, respectively. Based on the results of threshold study, a total of 8 rums were prepared by incorporating 0.05-0.25% and 0.3-1.8% *gurjo* stem- and betel leaf extracts, respectively, in pot-distilled alcohol of 40% abv. Sensory (appearance, flavor, taste, after-taste and overall acceptability) evaluations of the prepared rums were done by 9-point hedonic scale and data analyzed by ANOVA (using Genstat® 12.1.0.3338, a statistical software) to select the best rums (containing *gurjo* and betel leaf extracts, separately). Chemical analyses (fusel oil, methanol, total polyphenol, DPPH inhibition assay) of the best rum from each category (*gurjo*- and betel leaf) were also carried out.

Rum containing 0.15% *gurjo* extract was found to be significantly superior ($p<0.05$) in terms sensory quality. The fusel oil, methanol, total polyphenol and antioxidant activity were 143.5 mg/L, 33.52 mg/L, 12.07 mg GAE/L and 73.27% inhibition (DPPH assay), respectively. Similarly, rum containing 0.9% betel leaf extract was significantly superior in terms of sensory quality. The fusel oil, methanol, total polyphenol and antioxidant activity were 144.5 mg/L, 36.83 mg/L, 79.69 mg GAE/L and 67.92 % inhibition, respectively. The selected herbal rums had desirable sensory characteristics and the chemical parameters were within legal standard for rum. Thus, this product can also be beneficial for health.

Contents

List of Tables

List of Figures

List of Color Plates

List of abbreviation

Abbreviation	Full form
ABV	Alcohol by volume
ADY	Active dry yeast
ANOVA	Analysis of variance
AOAC	Association of official analytical chemists
DART-MS	Direct analysis in real time - mass spectrometry
DB	Dry basis
GAE	Gallic acid equivalent
GC	Gas chromatography
HPLC	High performance liquid chromatography
IS	International Standard
ISO	International organization for standardization
LSD	Least significance difference
MS	Mass spectroscopy
SD	Standard deviation
TPC	Total polyphenol content
TSS	Total soluble solid
WB	Wet basis
°Bx	Degree brix

Part I

Introduction

1.1 General introduction

Rum is a distilled alcoholic beverage which is made up of microbial fermentation of sugar cane molasses. It can also be made by fermenting sugar cane juice or syrup (Andrew and Piggott, 2003). Microbial fermentation is an important biotechnology process in the production of rum. Microorganisms that grow during this process in the production of rum. The microorganisms that grow during this process have a significant impact on the taste and quality of the final product. The basic process of making rum consists of the following operations: preparation of ingredients (molasses, sugar cane or sugar cane juice), fermentation of this material, distillation of this fermented product, collection of distillates, wooden aged barrels of distillate, and packaging of final products (Mangwanda *et al.*, 2021).

In the USA, rum defined as: "an alcoholic distillation made from the fermented juices of sugar cane, cane syrup, molasses, or other by-products of the sugar plant, produced at less than 190° proof in such a way that the distillate has the taste, aroma and characteristics normally attributed to rum, and is bottled at not less than 80° proof; and also includes only mixtures of such distillates" (Anon., 2010). According to the European definition rum is a spirit drink made solely by alcoholic fermentation and distillation, with less than 96% alcohol by volume (ABV), from either sugar cane sugar production or molasses or syrup obtained from sugar cane juice itself. The distillate is distilled to have certain recognizable organoleptic properties of rum (Communities, 1989). Rum production developed as a by-product of the cane sugar industry in the 16th century, mainly in the Caribbean and West Indies.

According to Ambrose *et al.* (2016) a herb is any plant whose leaves, seeds, or flowers are used for flavor, nutrition, healing, or perfumery. The main difference between herbs and spices is that spices come from different parts of the plant than the leaves, whereas herbs always come from the leaves. Herbs and spices have played an important role as flavorings, food preservatives and medicines for centuries. Studies on their health benefits have increased significantly in recent decades, as many herbs and spices are known to have properties associated with reducing the risk of chronic illness. The potential health benefits of herbs and spices include protection against cardiovascular disease, neurodegenerative

diseases, chronic inflammation, cancer, obesity and Type 2 diabetes (Vázquez-Fresno *et al.*, 2019).

This newly discovered love for alcoholic beverages stems from the fact that these new alcoholic beverages are herbal-based. Therefore, they are advertised as having medicinal properties that are not limited to health and sexual vitality. Therefore, it is very attractive to consume these herbal-based alcoholic beverages (HBAB) (Biney *et al.*, 2020).

Tinospora sinensis (*gurjo*) is a widely accepted herbal medicine employed solely as therapeutic drugs in South Asia, with a plethora of pharmacological processes. This plant is also known as *guduchi* or *amrita*. Based on the findings obtained from Ayurveda and ethnobotanical studies, *Tinospora sinensis* possesses a vast array of pharmacological applications (Singh *et al.*, 2003). Several extracts of *Tinospora sinensis* like aqueous, alcohol, methanol, chloroform, ethanol, acetone, etc. are chiefly used in pharmaceutical, pre-clinical and clinical trials. Alkaloids, glycosides, steroids, phenolics, flavonoids, saponins, aliphatic chemicals, and polysaccharides are among the active ingredients in this herb. Antioxidant activity is provided by each of these substances (Onkar *et al.*, 2012). *Tinospora sinensis* stem is effectively used in health care management, mostly in general fatigue, dyspepsia, fever, urinary disease, constipation, burning pain, diarrhea, blood accumulation, and jaundice treatments. It is also recognized that *Tinospora cordifolia* has immunomodulatory characteristics (Modi *et al.*, 2021).

The leaf of betel is an evergreen, perennial, climbing plant, with a heart-shaped ball and a white kitten. It has light yellow aromatic essential oils and has a strong flavor. It is grown in the tropics and subtropics, and its evergreen leaves are used for religious activities i.e. pooja and as a chewing stimulant. Piper plants are also used for many other purposes, such as food and spices, bait, fish poison, hallucinogens, insecticides, oils, decorations, perfumes, etc. It has spicy taste (Rai *et al.*, 2019). The importance of betel leaves has been described in ancient books of Ayurveda. The use of betel leaves was known for centuries for its curative properties. In Chinese folk medicine betel leaves are used for the treatment of various disorders and claimed to have detoxification, antioxidation, and antimutation properties (Toprani and Patel, 2013).

1.2 Statement of the problem

This study could be the first step in investigating the potential uses of locally available herbs as flavors, colors, nutrition, and medicines for producing rum in the industry. Rum is an alcoholic drink produced by the distillation of the microbial fermentation of molasses. Rum production developed as a by-product of the cane sugar industry in the 16th century, mainly in the Caribbean and West Indies. Initially inferior as a cheap distillate made and consumed by sugarcane plantation slaves, this drink has been associated with low socio-economic status for centuries (Mangwanda *et al.*, 2021). In rum, low amount of flavoring compound and medicinal compound are found, by fulfilling this problem, this research can enrich the medicinal value in rum as well as antioxidant and flavoring source. *Gurjo* has thousands of medical values. It gives a mild color. The leaf of betel has medicinal as well as flavoring but the color is greenish yellow. In addition, it enhances the taste, body, flavor, color and overall acceptability in the rum. The caramelized color may or may not be added. This helps to improve the attractiveness of rum. Thus, it can compete with other rum types available in the market.

1.3 Significance of the study

Different herbs like *gurjo* and betel leaf have been used as medicine since ancient periods. Usually, these natural medicines are not taken directly because of their strong intensity to affect the health. They need carrier for easy absorption and for proper work. Rum can be a very good carrier for these herbs. In addition, incorporation of herbs in rum may make the rum more palatable. It may also intensify the color, taste, and aroma.

1.4 Objectives

1.4.1 General objective

The general objective of the dissertation work was to prepare rum mixed with medicinal herbs (*gurjo* and betel leaf) and to conduct its quality evaluation.

1.4.2 Specific objectives

To fulfill the general objectives, the specific objectives undertaken were as follows:

1. Preparation of extracts from gurjo stem and betel leaf.

2. Threshold study for finding out the amounts of herb extracts that can be used in rum.
3. Preparation of herbal rum.
4. Sensory and physicochemical analysis of the prepared herbal rum.
5. Optimization of herb extract in the preparation of herbal rum.

1.5 Limitations of the study

1. Only one variety of each herb was studied during the work.
2. Ageing and other properties that may change with time were not studied due to time constraints.

Part II

Literature review

2.1 Rum

2.1.1 Background

Rum is a distilled alcoholic beverage made from sugarcane juice, sugar, cane syrup, or molasses (Green, 2015; Ickes and Cadwallader, 2018). The etymological origin of the word "rum" is unclear, with some authors suggesting that the term derives from "Saccharum", the common name for the sugarcane plant (*Saccharum officinarum* L.). Another derivative may come from the English slang word "rumbustion," which means "fuss or fuss." A more likely origin is "rumbullion", a term used to describe a drink boiled from the stem of the sugar cane plant. However, both terms were first reported around the same time as the origin of the word "rum" itself (Medeiros *et al.*, 2017). Despite the uncertain origin of the word, it was documented in 1654 within a law that was passed in Connecticut confiscating “Barbados liquors, commonly called Rum, Killdevil, or the like.” As this law showed, not only was the origin of the word ambiguous but there were multiple names by which the rum beverage was known, including kill-devil, brebaje, ron, Nelson’s Blood, grog, rumbullion, taffia, guildhive, Demon Water, Pirate’s Drink, Barbados water and Navy Neaters (Thompson, 1992).

2.1.3 Types of rum

In general, grades or varieties of rum are labeled with the aid of using their origin, flavor, shade, and taste. Numerous varieties of rum were advanced across the globe, inclusive of white, dark, amber, over-evidence, and spiced rums. Dark rum is elderly for over years in charred oak barrels and has a darker shade because it isn't filtered following the getting old method. Gold or ‘amber’ rum is likewise elderly in charred oak barrels, albeit for a shorter length of time (18 months). Caramel can be introduced after the getting old method to offer a greater shiny golden shade and regulate the shade of the rum to a predetermined standard (Murtagh, 2003). White rum (also known as ‘silver’, ‘light’ or ‘clear’ rum) is usually stored in stainless steel containers or casks and aged for 1-2 years (Nicol, 2003), using carbon filters after maturation to remove all colors and impurities process. It has a lighter taste than amber

and dark rum; As such, it is typically drunk in cocktails rather than neat. Over-protected rums are the most popular rums on the Caribbean Islands market (Pounder, 2010), with alcohol levels higher than the typical 37-40% alcohol per volume(FSANZ, 2000; Fahrasmane and Parfait, 2003). These rums make up almost 70–80% ABV and are generally used for punch (for making rum-based fruit cocktails). Spiced rum is enriched with spices such as cinnamon, anise, ginger, rosemary, or pepper in concentrations of up to 2.5% w/v during the mixing phase. Spiced rum is usually dark in color, with occasional sugar or caramel added for sweetness.

The types of rum are shown in Table 2.1

Table 2.1 Types of rum

Rum Type	Aging	Principal Production Region(s)	Ethanol Content (% ABV)	Additional Notes
White	Stainless steel casks (1–2 yrs.); often aged less than other rums	Puerto Rico	37–43	Lighter bodied; filtered before sale
Dark	Charred oak barrels (2 yrs)	Jamaica, La Martinica	37–43	Darker, fuller flavor
Amber/Gold	Charred oak barrels (1.5 yrs)	Cuba, Puerto Rico	37–43	Flavor not as complex as dark rums
Over proof/ Naval	Variable; can be sold with no aging in some countries	Jamaica	70–80	
Spiced	Charred oak barrels (1–2 yrs)	Jamaica, Puerto Rico	37–43	Most are darker in color and based on gold rums
Demerara Rum	Longer aging	Guyana	37–43	Distilled in old stills; complex flavor similar to Jamaican rum

Source: Mangwanda *et al.* (2021)

2.1.4 Rum production process

The production of rum follows the same basic procedures as many other spirits, i.e., raw material preparation, fermentation, distillation, maturation and bottling. Fig. 2.1 shows the flow chart for rum preparation.

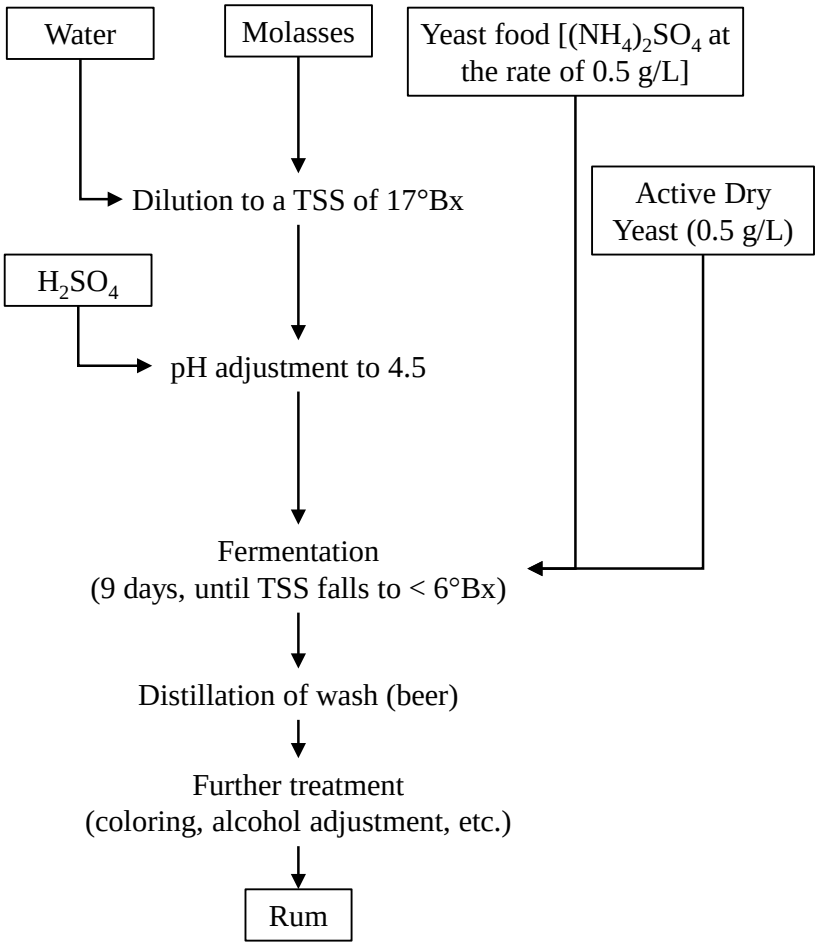

Fig. 2.1 Rum production process

Sources: Rai and Subba (2016) and Yildirim (2021)

2.1.5 Ingredients in rum production

2.1.5.1 Molasses

Molasses is probably the cheapest ingredient for ethanol production. Since it is a by-product of sugar factories, it is available in all sugar regions and this product has international trade. Molasses has many uses, from beverages, glycerin, acetic acid, baker's yeast, and lysine to fermentation, animal feed ingredients, and even fertilizers. The composition of molasses varies greatly depending on the non-sucrose in the raw liquor and the processing technique. However, sugarcane molasses can be considered as approximately 75-85% total solids, 30-36% sucrose, 10-17% (fructose + glucose), 10-16% ash, and small amounts of polysaccharides, oligosaccharides, organic acids, proteins, and nitrogen compounds. Since it contains about 50% fermentable sugar, it is suitable as a starting material for the fermentation process (Carioca, 2011).

Molasses is manufactured from sugarcane and beet molasses. Sugarcane is one of them that is used frequently and in large quantities (Clarke, 2003). Molasses is made by the separation of sucrose crystals after the water has evaporated from the juice (from sugar cane or beet) clarified during the production of granulated sugar. Concentrated juice promotes sucrose crystallization. Sucrose crystals are removed by centrifugation and the remaining viscous liquid is molasses. Molasses can be further recycled in this process to maximize sugar production. As a rule of thumb, the more recycling steps that molasses passes through, the lower its quality as a raw material for fermentation (Lino *et al.*, 2018). Some common terms for molasses are blackstrap molasses, high test molasses, and refinery molasses. Blackstrap molasses is a by-product of sugar cane mills or raw sugar refineries. This is a heavy, thick, viscous liquid that remains after the final stages of sugar crystallization, making it impossible to economically crystallize sugar using traditional methods. High-test molasses is the product obtained by concentrating purified cane juice to about 85 °Brix; it is partially alternated with an acid or enzyme invertase. High-test molasses is produced from cane juice instead of sugar, which is not a by-product of sugar production (Clarke, 2003). Refinery molasses comes from an intermediate stage of sugar production. Because it contains significant amounts of crystalline sugar, refineries do not sell it (Rai, 2012). Blackstrap molasses is the most commonly used molasses in rum production due to its availability and low cost (Clarke, 2003).

2.1.5.1.1 Physical and chemical properties of molasses

Molasses is a sweet, black, viscous liquid that stays after the sugar has been extracted. The physical properties of molasses vary depending on the composition. Viscosity can vary to several degrees depending on the inorganic and polysaccharide composition and temperature. Molasses has an acidic pH, usually between 5 and 7. The salt content (2-8%) can assist upward push buffer ability, stabilize taste, prevent hydrolysis, and offer a taste for farm animals' feed (Clarke, 2003). The composition of molasses varies greatly depending on the non-sucrose in the raw liquor and the processing technique. However, sugarcane molasses can be considered as approximately 75-85% total solids, 30-36% sucrose, 10-17% (fructose + glucose), 10-16% ash, and small amounts of polysaccharides, oligosaccharides, organic acids, proteins, and nitrogen compounds. Since it contains about 50% fermentable sugar, it is suitable as a starting material for the fermentation process. In addition to water and these sugars, it contains small amounts of many other compounds, such as; nitrogenous substances, phosphorous, metal ions, vitamins, gums, and colloidal constituents (Alcarde *et al.*, 2011).

As noted previously, molasses is wealthy in fermentable sugars and those are the primary chemical additives of this raw material. Traditionally, distillers have used °Brix as a dimension of sugar content material in molasses and molasses quality. For manufacturing of excellent pleasant rum with proper flavors and ethanol yield, molasses with a °Brix of 87.6 has been recommended. Lesser pleasant rums are acquired from molasses with °Brix much less than 85.4 and °Brix extra than 88.2 (Piggott *et al.*, 2003). However, the °Brix value now no longer gives a correct correlation with overall sugar content material due to the fact it's measure a degree of soluble solids and molasses consists of many soluble solids that aren't sugars. Total fermentable sugar is effortlessly quantified with the aid of using excessive overall high-performance liquid chromatography (HPLC) and such measurements will be the foundation for higher excellent grading of molasses than °Brix (Nicol, 2003). Molasses selection is critical in order to obtain efficient fermentations with high levels of ethanol, and the production of rum with a desirable flavor profile. The fermentation medium of molasses needs to contain appropriate levels of nitrogen, phosphorus, vitamins and minerals in addition to fermentable sugars (Alcarde *et al.*, 2011).

Molasses has a pH of 5.0 to 5.5 due to the presence of numerous organic acids, the most prevalent of which are acetic, malic, lactic and citric acids. Total nitrogen represents no more than 1.5% of molasses and consists of free amino nitrogen (ammonia and amino acids) and crude protein (about 3%). The gums of molasses can constitute up to 6% and are represented by hemicelluloses, pectins and dextrins which are found in sugar cane, and levans that may be produced by bacteria during the sugar cane milling process (Schoonees and Pillay, 2004).

Several vitamins have been found in molasses, with inositol and pantothenic acid being the most prevalent. Many factors affect the composition and quality of molasses, and these include the soil type, ambient temperature, moisture, season of production, cultivar and cultivation of sugar cane, the sugar refining process and conditions of molasses storage (Sawyer, 2005). Thus, variation may be found in nutrient content, flavor, color, viscosity and total sugar content.

2.1.5.1.2 Preparation of molasses for use in fermentation

As mentioned previously, rum distillers generally store molasses in bulk quantities in wells or tanks, and draw from these supplies to prepare it for fermentation. Such preparation includes clarification, adjustment of pH, heating to inactivate microorganisms, dilution with water, addition of nutrients for yeast growth (Murtagh, 1974; Nicol, 2003). The following sections describe the operations for preparing the molasses for fermentation. Immediately prior to use in fermentation, molasses is clarified by a combination of chemical or physical processes to partially remove suspended solids. This is usually done by addition of flocculating agents and allowing the solids to sediment. Centrifugation may also be used to clarify the molasses. At this stage, the pH is adjusted to values around 5.0-5.5 by addition of sulfuric acid, and the mixture is given a mild heat pasteurization treatment (80°C). Duration of pasteurization is often unrecorded and as such will vary among distilleries. It is important to remove colloidal material from the molasses, otherwise it will cause severe fouling of the distillation columns, thereby leading to inefficiency of the stills and production downtime due to increased frequency of cleaning (Murtagh, 1995). After settling or centrifugation of the solid materials, the clarified liquid is pumped off, and then diluted with potable water to give a final concentration of 100-150 g/L for fermentable sugars (15 to 20°Brix). Yeast nutrients such as ammonium sulphate and vitamin mixtures may be added at this time to ensure complete fermentation (Paturau, 1969; Nicol, 2003).

2.1.5.2 Dilution water

Water is used to dilute the molasses for preparation of the fermentation medium. Waters used for dilution purposes may originate from various locations including town water, treated distillery waste waters, rivers and creeks and rainwater storage tanks. As such, the quality may vary greatly, and microbial testing and chemical analysis should be undertaken. Chemical testing should determine ion content, heavy metal presence and hardness. Microbiological testing for total viable count, coliforms and *E. coli* and *Clostridium perfringens* should be done as a minimum (Fahrasmane and Ganou-Parfait, 1998). Detailed testing procedures and results are difficult to find in the rum literature as water testing has been deemed more important for dilution of the finished matured spirit rather than that used for dilution of fermentation medium. Arroyo (2015) recognized the importance of using good quality water, during dilution of molasses, to limit the potential production of off-odors by contaminating microflora during subsequent fermentations. His research also discussed the potential of increasing the mineral content of molasses because of its effect on yeast growth and activity.

2.1.5.3 Yeast inoculum for fermentation

Modern rum distilleries conduct fermentation by inoculation with starter cultures of selected strains of the yeast, *Saccharomyces cerevisiae*. These strains may be maintained as pure cultures “in house” and propagated to inoculum volumes as needed on site. Some distillers may purchase their yeast as active dry cultures from specialized companies and rehydrate them for direct inoculation into the fermenters according to the manufacturer’s instructions. The process for yeast propagation on site is briefly outlined here and described in Pontes *et al.* (2006).

Yeast propagation is initiated by preparing an inoculum from a stock culture of the selected yeast strain. This stock culture will be maintained on site and securely stored in established culture collections for retrieval as needed. The purity of the culture is verified by agar plating, from which a small volume (100-500 ml) of liquid culture is prepared under strict aseptic conditions. This culture is used to inoculate about fifty liters of sterile medium (autoclaved) and incubated with aeration to increase the numbers of growing cells prior to aseptic transfer of this culture to a larger volume (500 L). Culture transfer to progressively larger volumes is conducted to provide a yeast inoculum that gives 10-30% of the final

molasses fermentation and a starting yeast population of approximately 10^6 cells/ml (Murtagh, 1995).

The propagation medium is usually similar to the molasses fermentation medium (with yeast nutrient addition) to ensure that the yeast is well adapted to that condition. While medium used in the early stages of propagation can be sterilized by autoclaving, the final stages of propagation usually require large volumes of molasses medium that is not autoclaved and has been processed in a manner similar to the final molasses fermentation medium to contain about 10-15% fermentable sugars. Glucoamylase and possibly yeast foods are also added to the propagation medium; however, few guidelines are available. The temperature at which propagation is conducted is normally monitored and controlled. There are variations in temperature from plant to plant (depending on yeast strain). Generally, propagation is conducted at a temperature at least 2-5°C below that of normal fermentation temperature. Once the yeasts have entered their active stage of growth (log phase), they are transferred from the propagator into the batch fermenter. There are typically 4 different propagation systems that can be used in industry; continuous, semi continuous, multiple batch and single batch (Nicol, 2003).

Throughout propagation, quality control measures should ensure cell viability and culture purity. Yeast cell viability can be quickly determined using methylene blue (or other cellular stain) to differentiate between viable and non-viable cells. Counts performed in combination with a hemocytometer can give relatively quick approximations compared to cultural plating methods. Cultural purity can also be quickly determined using microscopy; however, cultural plating or real time polymerase chain reaction (PCR) can also be used (Simmomds, 1994).

2.1.6 Fermentation

Fermentation by microorganisms, principally yeasts, is the key operation in rum production. The profile of flavor volatiles that distinguish rum from other distilled alcoholic beverages is produced during the fermentation of molasses by the microorganisms that grow. Without this fermentation, there would be no rum. Yeast conducts an alcoholic fermentation of the sugars in molasses, metabolizing them into mainly ethanol and carbon dioxide, and a vast array of small amounts of secondary end products. In some cases, bacteria may be associated with the fermentation (Lehtonen and Suomalainen, 1977).

In earlier times, fermentation was conducted in large, open concrete tanks or large wooden vessels. Today, most rum fermentations are conducted in large (up to 100,000L) stainless steel closed “cyclindro-conical” vessels. These vessels are equipped with stirring and sparging devices, temperature control, and cleaning in place (CIP) facilities (Broom, 2003). Although the fermentations may be gently stirred to keep the yeast cells in suspension, they are not aerated.

Rum fermentations can vary in length from 24 h up to 10 days. Most distilleries run fermentations to a standardized time for each specific rum. Longer fermentations are used to produce the heavier flavored rums, while shorter fermentations (24 -30 h) are used to produce lighter style rums. Fermentations are generally conducted at 28-35°C to maximize their rate of completion and are considered complete when the desired alcohol levels (%) have been reached (approximately 5-7%). Some distilleries use change in final gravity or °Bx, from set up to determine the completion of fermentation; however, it should be noted that all three units are related (Destruhaut *et al.*, 1986).

Since the fermentation process generates heat, it is necessary to cool the fermenters, so the temperature does not exceed 37°C. At temperatures exceeding this value, the yeast becomes sensitive to the increasing levels of ethanol and may be inactivated. If this occurs, the fermentation will stop and remain incomplete or “stuck”. Such occurrences lead to major inefficiencies. Temperature control is needed to ensure that the temperature limits are not violated and that yeasts are not killed in the process. Cooling can be provided to help control temperature fluctuations. This can be in the form of; internal cooling coils or panels, double jacketed walls with cooling in the outer walls, recirculating spirals, plate and frame or shell in tube heat exchangers (EAC, 2001).

2.1.7 Distillation of ferment

Distillation is a critical process in rum production that separates, concentrates and selects the volatile components of the fermented molasses (Bluhm, 1983). The volatile fraction of the fermented molasses consists predominately of ethanol, and lesser amounts of higher alcohols, organic acids, esters, phenols and some carbonyl and nitrogenous compounds. The use of the collective term “congeners” has been applied to describe all volatile components of rum other than ethanol (Nykaenen and Suomalainen, 1983).

Distillation is used to capture most of the ethanol and refine flavor by selecting for the types and concentrations of other, desirable volatile compounds; however, it does not create these base components. Creation of the desirable flavor volatiles occurs mainly during the fermentation of molasses, but some may occur in the molasses before fermentation (Yokota and Fagerson, 1971). Distillation can produce new compounds via esterification, dehydration etc. from the base components produced in fermentation. The use of pot distillation is known to increase furfural concentration (Madrera *et al.*, 2003).

Distillation is based on the principle that different components within a liquid mixture, such as the fermented molasses, have different temperatures at which they boil and transform to a vapor or gaseous phase. Heat is applied to the liquid mixture. Simply, the smaller, more volatile components are vaporized and boil off first and are progressively followed by the less volatile components. Non-volatile substances are left in the liquid mixture. As the temperature of the vaporized fraction is decreased, the individual components revert to their liquid phase and can then be collected in this state. This process of transformation back to the liquid phase is called condensation (Paine and Dayan, 2001).

The basic apparatus for distillation consists of a vessel (still) in which the liquid mixture is heated. The base of the vessel is attached or connected to columns, into which the volatiles vaporize and eventually condense back to a liquid, and capture vessels for collecting the condensed liquid (distillate). The columns may be differentially cooled to encourage condensation (Kampen, 1975).

Distillation efficiency can be increased by the addition of a reflux step. This is a method of returning a proportion of the condensed distillate back into the distillation column. The down flowing reflux liquid enters the column and cools. It condenses the rising vapors and works to increase the separation efficiency of the distillation column. Increasing the amount of reflux for a column will improve the separation of lower boiling components from higher boiling compounds, resulting in a distillate with a higher composition of a desired product (Wankat, 2007).

Separation of the volatile compounds is based on volatility differences and occurs through heating (and cooling). The degree of separation of the desired component may be affected by various operating conditions such as pressure, temperature, the initial feed composition

and liquid phase conditions. Controlling the distillation process is crucial for the production of product with consistent and desirable quality (Nicol, 2003).

Two types of distillation processes are used in the production of rum: batch distillation and continuous distillation. Batch distillation, in pot stills, is used to manufacture rums with stronger, heavier flavors such as those of Barbados, Bermuda, Jamaica and other English-speaking regions in the Caribbean. Continuous distillation, using column stills, is used for the production of lighter style rums of the former Spanish colonies (such as Cuba, Panama). Some distilleries use a combination of both techniques (Green, 2015).

2.1.7.1 Batch distillation

Pot stills are the earliest known distillation apparatus and, until the mid-1800s, all rums were produced by this process. Pot stills consist of three parts; the kettle (boiler), condenser and gooseneck, similar to those used in whisky distillation. The kettle is the base of the vessel into which fermented molasses is transferred and heated by steam injection, either directly or indirectly through heating coils. Low boiling components, including ethanol, will begin to vaporize and pass through the gooseneck into the condenser (or retort depending on still). Limited reference is made to specific temperatures throughout the literature. This may have been influenced by two factors. Traditionally pot stills are heated by steam or fire, making temperature difficult to control. Distillers wishing to keep production practices secret from competitors may also have some bearing on the lack of records (Andrew and John, 2003).

The liquid distillate obtained from this type of process is also known as “single distillate” since it is processed through the still only once, giving a product of about 40-60% alcohol by volume. This process gives a heavy pot still rum. Typically, however, this liquid is processed a second time, thus producing a double distillate which is cleaner and stronger than the single distillate. This re-distillation enables further separation of the desirable volatile compounds. This occurs due to the increased ethanol concentration of the primary distillate compared to the molasses fermentation. This increase in ethanol concentration decreases the boiling temperatures, thus ensuring greater variation as to when different volatile compounds are liberated into the vapor phase. The process can be repeated several times, thereby obtaining a cleaner, stronger more rectified spirit each time. Distillation is performed batch by batch and is very labor intensive. Pot distillation is usually performed in

conjunction with the addition of dunder in the fermentation, thus producing a heavy, high ester rum (Nicol, 2003).

The first fractions (first 5 min of process) to be collected as distillate contain about 88% alcohol by volume (ABV), but also contain some pungent less desirable flavor volatiles. This fraction, often referred to as the first cut or low wines, may be discarded. The final fractions of batch distillation will contain much less ethanol (less than 40-45% ABV) as it has already been distilled out, along with other volatiles with less desirable flavor attributes. Such fractions are often referred to as late cuts, tails or feints, and may also be discarded. The "center" fraction (also called "hearts" or "spirit" or "middles") usually contains the most desirable flavor volatiles and is the cut that is collected (85% ABV at the beginning). As distillation proceeds, the concentration of ethanol in the distillate decreases. Generally, collection of the "hearts" is stopped when the alcohol content of this fraction is about 40-43% ABV (Nicol, 2003; Piggott and Conner, 2003)

2.1.7.2 Continuous distillation

Continuous distillation gives a distillate with more consistent composition than batch distillation. The distillation column or tower consists of two sections. The portion of the tower above the molasses feed entry point is defined as the 'rectifying section' of the tower. The part of the tower below the feed entry point is referred to as the 'stripping section' of the tower. Throughout the column there are a number of horizontal trays placed at different levels. Pre-heated fermented liquid is usually introduced at the top (or at least half way up the column) (Murtagh, 1974; Wankat, 2007).

As the liquid makes its way down the column, it is heated by rising vapor. This liquid-vapor contact occurs on the horizontal trays which commonly have holes punched through the metal, or specialized "bubble caps" which also allow for liquid-vapor contact. As the liquid flows on to the tray, the rising vapor is forced to come in contact with it. During this contact, heat is exchanged, and the more volatile components tend to concentrate into the vapor. After repetitive liquid-vapor contacts over the height of the column, the most volatile compounds rise to the top of the column. This partial separation allows column distillation units to be more efficient at separating fermentation components than pot stills. Once the fermented medium reaches the bottom of the still, it contains no alcohol and is removed, as dunder, through a release valve. Careful control of the heating rate of the column, and the

degree of reflux in the rectifying section, allows column operators to dictate the ethanol concentration of the primary distillate taken from the column (Murtagh, 1995; Wankat, 2007). The highest temperature (usually in excess of 90-95°C) in the tower will occur at the base, and the temperature in the tower will regularly and progressively decrease from the bottom to the top of the tower. To produce the temperature variations, reboilers (heat exchangers) are often used to heat and partially vaporize the liquid streams in the lower sections of the column (Wankat, 2007).

The rectifying sections of both pot stills and continuous columns are often under reflux, which is where condensed liquid collecting near the base is continually pumped and fed back into the rectifying section near the top. It then simply combines with the liquid phase flowing down through the column. This enhances the interaction of the vapor and liquid phases and achieves greater separation of the volatile components. Distillation systems for rum production usually operate as two separate processes: primary distillation and secondary distillation (Wankat, 2007).

Primary distillation operates similarly to pot distillation, generally referred to as “low wines” with a concentration of approximately 50% ABV. Primary distillation allows subsequent distillations (secondary distillation) to achieve greater separation of the volatile compounds. Unlike primary distillation, the distillate obtained from the secondary distillation is collected in fractions. These fractions are either based on the collection time or the ethanol concentration of the distillate collected from the rectifying section. If sufficient separation of the volatile components is achieved, each fraction should contain significantly different flavor profiles. The first fraction, rich in highly volatile components, is called the heads or ‘high feints’ and is usually discarded because it contains high concentrations of aromatic esters and acids. The second fraction is the final rum distillate or ‘raw rum’ or ‘hearts’ which is only collected up to a certain point, once again based on either collection time or percentage of ethanol, depending on distiller. Collection of distillate beyond this point is undesirable as fusel oils constitute a large proportion of the less volatile components (Nicol, 2003; Wankat, 2007).

2.1.8 Aging and maturation

Freshly produced rum distillate has some strong, raw flavors that are not appreciated by all consumers. Consequently, the fresh distillate can be subjected to a process of maturation

where it is stored in large wooden barrels, generally made of oak, that hold approximately 120 – 150 L (QuesadaGranados *et al.*, 2002). Acetate esters of higher alcohols will hydrolyze more rapidly than the corresponding ethyl esters, independent of the alcohol content of the beverage. It is important to note that some provincially produced rums are not subject to ageing and are available for consumption immediately after distillation (Broom, 2003). During maturation, a range of physical and chemical interactions takes place between the barrel wood, the surrounding atmosphere and the maturing spirit. These interactions transform both the flavor and composition of the alcoholic beverage. The effects and time required for maturation are variable and are influenced by a wide range of factors, particularly the type of barrel used (Kampen, 1975; Nicol, 2003).

Maturation is not the same as ageing. Maturation is the end stage which is reached after ageing (the means to obtain maturation). A mature rum is not defined as one that has spent a fixed period of time in a barrel. Rather, maturity is measured by the rum possessing distinctive characteristics acquired during ageing. Such characteristics are body, color, aroma and taste. Distillate straight from a still is clear but maturation in wooden barrels gives the finished rum its yellow/golden color, depending on how long it is aged. Ageing is simply the time the rum is stored in the wooden barrel (Nicol, 2003).

There is a diversity of chemical reactions that may occur during maturation. These include (Broom, 2003):

1. Direct extraction of chemical constituents from the wood
2. Decomposition of oak on a molecular level and interaction of resulting compounds with the distillate
3. Reactions between the constituents extracted from the wood and those in the rum distillate
4. Reaction between wood compounds within the raw rum, reactions between raw rum compounds
5. Evaporation of volatile compounds through the cask
6. Interaction between the raw spirit and air present in the cask/ vat.

The length of time that raw rum is aged depends on the type of rum being produced and also, to a certain extent, the market in which the rum will be sold. “White” rums, those that are clear and used as the basis for cocktails, are generally not aged for extended periods,

except for where the law requires a minimum ageing period. These rums are usually aged in old, well used barrels and are submitted to charcoal filtering to remove any color prior to bottling. Amber and dark rums are typically aged for a period of time between 12 months and 25+ years. Typically, the longer the rum is in oak, the darker the rum (Broom, 2003).

The rum industry uses two main types of barrels: those which have already been used in curing whisky and new oak barrels. The main reasons for using pre-used whisky barrels is that they are cheaper than new barrels and they have previously been 'cured' or charred (Jeffery, 2012).

Cask/barrel manufacture is not standardized, with large discrepancies between American and European cooperages. Barrels are very expensive and in high demand, consequently, rum distilleries may buy old barrels that have been used for the maturation of other alcoholic beverages, such as whisky, wine and brandy. Barrels are heated or charred (burning of the inside surface of the barrel) prior to raw spirit being stored. This charring changes the physical and chemical composition of the wood by caramelizing sugars, increasing vanillin and helping to extract tannins (Mosedale, 1995; Broom, 2003).

The quality of ageing barrels is a very important parameter in the production of rum. Distillers, however, have little control over some of the factors affecting this quality such as: character of the soil and climate in which the trees were grown, age of the trees when cut, manner in which they were cut, part of tree from which the staves were derived, variety and amount of resins present, and period of ageing given to the boards prior to barrel construction (Mosedale, 1995).

Many countries have legislation governing the minimum ageing time which must occur prior to a product being sold as "rum". For example, to be sold in Australia, the Dominican Republic and Panama, rum must be aged for a minimum of 2 years. Mexico legislation requires a minimum of 8 months. However, some countries are less stringent and allow rum to be sold without any ageing such as rhum agricole and Brazilian cachaça (Broom, 2003).

2.2 *Tinospora sinensis* (*gurjo*)

Tinospora sinensis (Lour.) Merr. belongs to the Menispermaceae family and is known in India by different names such as *giloy*, *guduchi*, and *amrita* (Mahima *et al.*, 2014). It is well known in Ayurveda and traditional medicine for its admirable therapeutic efficiency

(Sankhala *et al.*, 2012). Because of its capacity to provide freshness, energy, and long life, it is known as the "Nectar of Immortality" (Upadhyaya *et al.*, 2011). *Tinospora sinensis*, which grows on neem trees (*Azadirachta indica*), has a high medicinal potential and is known as '*neem giloy*' because of its synergistic impact (Mittal *et al.*, 2014). Since the beginning of time, plants have been important to people as a source of food and medicine. The popularity of medicinal plants is rising at the moment due to the various phytochemicals that are useful for therapeutic purposes (Rani *et al.*, 2015).

The stem is a more widely used and beneficial component of the plant than the leaves (Sarala *et al.*, 2012) and its extract has been shown to be a good source of antioxidant for nutraceutical purposes, providing protection against cardiovascular disease, premature aging, and cancer (Ilaiyaraja and Khanum, 2011). Traditionally, *Tinospora sinensis* (Lour.) or Merr. leaf and stem juice have been used to cure chronic rheumatism, ulcerated sores, and piles by disinfecting the fresh stem and leaves (Rajgopal *et al.*, 2013). The Newar community in Kathmandu and far west Nepal are seen to use the juice, powder, or liquid of *Tinospora sinensis* (Lour.) Merr. for diabetes and gastritis (Balami, 2004).

In China, the plant is used to relieve wind dampness, stimulate blood circulation, relax joint and muscle, relieve pain, and reduce swelling (Xu *et al.*, 2010). In India's North Central Western Ghats, the stem decoction of *Tinospora sinensis* (Lour.) Merr. is used to cure bone fractures and strengthen bones (Upadhya *et al.*, 2012). *Tinospora sinensis* (Lour.) Merr. has been reported to be used as a substitute for *Tinospora cordifolia* (Willd.) Miers (Khare, 2007). The common names and taxonomic classification of *gurjo* according to Sharma *et al.* (2010) is shown in Table 2.2 and Table 2.3.

Some of the medicinally important species are *Tinospora cordifolia , Tinospora sinensis, Tinospora crispa, Tinospora cordifolia, Tinospora malabarica, Tinospora tomentosa, Tinospora uliginosa,* etc. (Neeraja and Margaret, 2013).

Table 2.2 Botanical names of *gurjo*.

Language	Botanical name
Sanskrit:	*Guduchi, amrita*
Bengali:	*Giloe, gulancha*
Hindi:	*Giloya*
Gujarati:	*Gado, galo*
Telugu:	*Duyutige, teppatige*
English:	Heartleaf moonseed
Malayalam:	*Amruthu, Chittamruthu*
Tamil:	*Shindilakodi*

Sources:Biswasroy *et al.* (2020) and Sharma *et al.* (2010)

Table 2.3 Taxonomic classification of *gurjo*

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Ranunculales
Family:	Menispermaceae
Genus:	*Tinospora*
Main species:	*Tinospora cordifolia*
	Tinospora sinensis

Sources: Meshram *et al.* (2013) and Sharma *et al.* (2010)

2.2.1 Plant morphology

Tinospora is a large, deciduous, extensively spreading and climbing shrub with several elongated twining branches (Upadhyay *et al.*, 2010). Different parts of exhibits different types of morphology which are as described in the following sections.

2.2.1.1 Root

Roots are aerial, threadlike, long filiform, threadlike, squarish, and extend downward from mature branches or cut parts of stems, sometimes reaching the earth by continuously lengthening (Spandana *et al.*, 2013). The dried aerial roots have a light grey-brown or creamy white color, are odorless, and have a bitter flavor. Starch can be found throughout the aerial root's parenchyma.

2.2.1.2 Stem

The stem of this plant is succulent, with long, filiform, fleshy, and climbing tendencies. The branches give rise to aerial roots. The dried stem is slender, cylindrical, and somewhat twisted. The outer bark is thin and papery, and the color ranges from brown to greyish. Transverse sectioning of the stem reveals a wheel-like structure. The stem powder has a characteristic aroma and bitter flavor and is creamish brown to dark brown in color. The stem is used to treat dyspepsia, fever, and urinary tract infections. The starch extracted from the stem, known as "*guduchi-satva*" is extremely nutritious and digestive, and is used to treat a variety of conditions (Bishayi *et al.*, 2002).

2.2.1.3 Leaves

The leaves of this plant are membranous, simple, alternate, with long petiole approximately 15 cm which is round, pulvinate, heart shaped, twisted partially and half way round (Nasreen *et al.*, 2010). In the beginning, the leaves are a bright green color, but as they age, they turn yellowish green to yellow. The leaves are harsh and have an unremarkable odor. The lamina is ovate-cordate, measuring 10-20 cm long and 8-15 cm wide. Protein, calcium, and phosphorus are abundant in leaves (Sharma *et al.*, 2010).

2.2.1.4 Flowers

The flowers are tiny and unisexual, and the color is a greenish yellow. Male flowers are grouped together, while female flowers are found alone. There are six sepals in each of the two series of three. The outer sepals are smaller than the inner ones. Petals are six in number, smaller than sepals, free-floating, and membranous. Summer is when flowers bloom (March to June) (Sinha *et al.*, 2004).

2.2.1.5 Fruit

Fruits are fleshy, single-seeded, and occur in clusters of one to three. These are drupelets with a subterminal scar on a thick stem. The fruit is ovoid in shape, with a smooth texture and a scarlet or orange red color (Nasreen *et al.*, 2010).

2.2.1.6 Seed

The seeds are white, bean shaped and curved. Embryo also turned in to curve shape automatically (Singh *et al.*, 2003).

2.2.2 Chemical constituents and biological activity of *gurjo*

The chemical constituents and biological activities of *Tinospora cordifolia* are summarized in Table 2.4.

Table 2.4 Major and sub-groups of natural products present in different parts of *Tinospora cordifolia* and their biological activities.

Active component	Compound	Plant part	Biological activity
Alkaloids	Berberine, Choline, Palmatine, Tembetarine	Stem, Root	Anti-viral infections, Anti- cancer, Anti-diabetes
Diterpenoid lactones	Furanolactone, Tinosporin, Isocolumbin	Whole plants	Anti-inflammatory, Anti-hypertensive, Anti-microbial
Terpenoids	Epoxy-clerodane diterpene	Stem	Anti-inflammatory
Glycosides	Cordifolioside A, Syrigin, Tinocordiside	Stem	Immunomodulation, Anti-cancer
Steroids	β–sitosterol, Makisterone A	Shoots	Anti-inflammatory
Sesquiterpenoid	Tinocordifolin	Stem	Antiseptic

Sources: Upadhya *et al.* (2010),Sundarraj *et al.* (2012) and Biswasroy *et al.* (2020)

2.2.3 Proximate composition of *gurjo*

The proximate study (Nile and Khobragade, 2009; Rahal *et al.*, 2014; Tyagi *et al.*, 2020; Modi *et al.*, 2021) revealed the presence of carbohydrate, protein, fiber, fat, and moisture which helps to determine the nutritive value of the medicinal plant *gurjo*. Nitrogen free extractives (NFE) vary from basic sugar to more complex compounds, like starch and fiber. The findings derived from the proximate assessment of the oven dried stem sample's nutritional values are summarized in Table 2.5.

Table 2.5 Proximate composition of dried *Tinospora cordifolia* stem

Proximate Composition	% In dried sample
Moisture content	10.01
Total ash	7.05
Crude fat	1.77
Crude fiber	26.99
Protein content	8.06
Carbohydrate	46.11

Source: Modi *et al.* (2021)

2.2.4 Ethnobotanicals and traditional uses of *gurjo*

Those species have been used for therapeutic purposes for a long time. According to research, they are employed as an adjunctive therapy in cancer and liver protection, as well as a treatment for oral, skin, respiratory, and urinary tract infections, ulcerations, and diabetes (Zhang *et al.*, 2010).

Ayurveda states that *Tinospora sinensis* has an unpleasant flavor that is bitter, pungent, and astringent. Even at the molecular level, the bitter flavor is considered to enhance metabolic activity. It has been shown to be effective in treating gastrointestinal conditions such dyspepsia, flatulence, gastritis, jaundice, diarrhea, splenomegaly, and hemorrhoids. It is currently more likely to be a research topic and plays a part in the treatment of metabolic

illnesses like diabetes and kidney disorders. It is prescribed for intermittent fevers, infective conditions, urinary disorders, skin diseases, and eye diseases. It is frequently used as an ingredient to treat gout and rheumatoid arthritis in combination with other herbs. Fractures are treated with the whole plant that has been properly ground. Additionally, *Tinospora sinensis* is regarded as a general tonic and has a positive impact on health equipped with a variety of nutritive ingredients (Panchabhai *et al.,* 2008). The numerous and significant ethnomedicinal characteristics that even this genus contains could serve as a foundation for further study into the phytochemical and pharmacological characteristics of this species.

2.2.5 Immunomodulatory activity of *gurjo*

Few studies have focused on the immunomodulatory abilities and mechanisms of *Tinospora sinensis. Tinospora sinensis* stem contains an ingredient known as arabinogalactan polysaccharide (G1-4A), which controls cytokines and nitric oxide excretion to protect mouse macrophages from endotoxic shock brought on by lipopolysaccharide (Desai *et al.*, 2007). Different polysaccharides, such as arabinose, glucose, and fructose, may be involved in *Tinospora sinensis* immunomodulatory action (Sharma *et al.*, 2012) and induces a nonspecific immune response (Alexander and Kirubakaran, 2010); however, the mechanism is poorly elucidated. Additionally, supplementing mice with *Tinospora sinensis* results in splenomegaly, an increase in macrophages, T cells, and B cells, as well as an increase in the production of antiapoptotic genes in immune cells (Raghu *et al.*, 2009).

Many active compounds, including N-methyl-2-pyrrolidone, N-formylannonain, 11-hydroxymustakone, Cordifolioside A, Tinocordiside, Syringin, and Magnoflorine, are found in Tinospora sinensis (Sharma *et al.*, 2012), usually show practical immunomodulatory and cytotoxic effects. It was reported that such active components could boost macrophage phagocytic properties by causing free radicals to be produced in human neutrophils (More and Pai, 2012).

2.2.6 Safety evaluation of *gurjo*

Tinospora herbs have a long history of use in medical contexts, which confirms their effectiveness as medicines. When used in tolerance, there have been no negative effects reported for this genus. For children and newborns, *Tinospora sinensis* is utilized as a growth booster. *Tinospora sinensis* has no negative effects in an acute toxicity investigation with a

dose of 3 g/kg, and the experimental rats did not die (Agrawal *et al.*, 2002). More and Pai (2012) suggested approaches and interventions from AYUSH to address COVID-19 health challenges. The prophylactic care and as add on to standard care for *Tinospora cordifolia* aqueous extract with a 500 mg twice a day (bid) for 15 days or 1 month (as directed by Ayurveda physician with warm water. The herb has been reported to be safe even in high doses for long term, clinical studies have also shown it to be safe in long term use.

When administered to rats in doses of 0.1 g/kg for a period of 12 weeks, *Tinospora sinensis* has no adverse effects on liver and renal function markers. Leukocytosis and neutrophilia increased in rats, but in healthy persons it had no effect (Panchabhai *et al.*, 2008). *Tinospora sinensis* treatment has no clastogenic or DNA-damaging effects on bone marrow erythrocytes and peripheral blood cells (Chandrasekaran *et al.*, 2009). And no neurological impairment or marked central nervous system depressant activities were shown (Karkal and Bairy, 2007).

Cytotoxicity was evaluated in terms of LC50 (lethality concentration). The result showed that the extract of *Tinospora cordifolia* was found to be toxic with an LC50 value of 232.64 µg/ml. The bioactive component present in the plants could be the result of its pharmacological effects that support the traditional use of plants (Shrestha and Lamichhane, 2021). The literature survey on hepatic effect by *Tinospora cordifolia* (TCF) on 6 male Wister rats with alcohol intoxication of duration on treatment 200 mg extract (water and sediment) /kg brew/day for 15 days shows significant improvements in the total protein content, the liver function tests and the lipid profile tests except for high-density lipoprotein cholesterol. Also, significantly lower levels of lipid peroxidation and glutathione (GSH). The liver histology showed improvements despite the collection of many lymphocytes within the hepatic parenchyma and the prominent Kuepfer cells (Chavan *et al.*, 2017). Also, the literature survey on hepatic effect by *Tinospora crispa* (TCP) on 1 man of duration on treatment 10 pellets/day for 4 weeks shows centrilobular necrosis with inflammatory cell infiltration compatible with a toxic etiology and complete recovery after discontinuation (Langrand *et al.*, 2014).

2.2.7 Medicinal applications of *gurjo*

Tinospora sinensis is widely used in traditional ayurvedic medicine in India due to its biological activities, which include anti-inflammatory, immunomodulatory, anti-oxidant,

anti-diabetic, anti-periodic, anti-spasmodic, anti-neoplastic, anti-stress, anti-leprotic, anti-malarial, hepato-protective, anti-allergic, and anti-arthritic activity, as well as various fevers, asthma, diabetes, dyspepsia, jaundice, urinary difficulties, skin illnesses, and chronic diarrhea and dysentery are all treated using *Tinospora sinensis* (Sharma *et al.*, 2012). Following are the medicinal applications of *gurjo*:

- The root and stem of *Tinospora cordifolia* an antidote to snake bite and scorpion sting (Zhao *et al.*, 1991).
- The stem is bitter, stomachic, diuretic, stimulates bile secretions, allays thirst, enriches the blood and cures jaundice (Nayampalli *et al.*, 1988).
- The juice of plant stem is useful in diabetes, dyspepsia, vaginal and urethral discharges (Singla *et al.*, 2010).
- The bark of this plant acts as anti-allergic, anti-spasmodic, anti-pyretic, anti-leprotic (Asthana *et al.*, 2001).
- The powder of root and stem is used along with milk for treatment of cancer (Bhatt and Sabnis, 1987).
- The whole plant of *Tinospora cordifolia* used in scabies in swine, diarrhea, urinary diseases, syphilis, skin diseases, bronchitis, to promote longevity, increase body's resistance and Stimulate the immune system (Kapur *et al.*, 2008).
- The dry stem crude extract of this plant which was poly saccharide in nature shows a polyclonal B-cell mitogen activity and active components of stem extract enhanced the humoral response in mice (Kalikar *et al.*, 2008).
- *Giloy* (*Tinospora cordifolia*) juice which is a mixture of *giloy* herb and *tulasi* (*Holy basil*)leaves is used against monkey malaria (Vashist *et al.*, 2011).
- The stem aqueous extract of *Tinospora cordifolia* shows anti-inflammatory effect in both acute a sub-acute model of inflammation (Jana *et al.*, 1999).
- It is used in treatment of jaundice because it reduces body heat (Sangeetha *et al.*, 2013).
- The stem of this plant regulates the blood sugar level due to the presence of alkaloids (Patel and Mishra, 2011).

2.3 *Piper betle* (*paan*)

Piper betle L. (synonym: *Piper betle* Blanco) (Piperaceae) is a widely known perennial creeping plant belonging to the genus Piperaceae and originates from central and eastern Peninsular Malaysia and is distributed to East Africa and tropical countries of Asia (Madhumita *et al.*, 2019). It is a commercial cash crop (Bajpai *et al.*, 2010).

The creeper plant has heart-shaped leaves with pointed apex (Durani *et al.*, 2017; Sarma *et al.*, 2018; Madhumita *et al.*, 2019). *Piper betle* leaves length can extend approximately to 18 cm and 12 cm in width (Pin *et al.*, 2009b). The leaves give off a sharp and pungent aromatic smell (Ravindran *et al.*, 2012; Chan and Wong, 2014; Sarma *et al.*, 2018). The stalk is smooth and long. The plant usually cultivated from 150 cm to 180 cm in height in plantation (Thamaraikani and Kulandhaivel, 2017). The height may grow up to 20 meters when left grow wildly without trimming and cutting (Chan and Wong, 2014). *Piper betle* preferred neutral soil between pH 7 to 7.5 as bed soil (Sarma *et al.*, 2018).

The common names and taxonomic classification of *paan* according to Azahar *et al.* (2020) is shown in Table 2.6 and Table 2.7.

Table 2.6 Botanical names of *Piper betle*.

Language	Local names
Sanskrit:	*Tambool, Mukhbhushan, Nagavalli, Varnalata, Nagavallari*
Hindi, Bengali, Urdu:	*Paan*
Telugu:	*Nagballi, Tamalapaku*
Tamil:	*Vetrilai*
Gujarati:	*Nagarbael*
Marathi:	*Vidyache pan*
Malayalam:	*Vettilakkoti, Vettila*

Continued …

…. **Table 2.6** continued

Language	Local names
Kannada:	*Veeleya, Veeleyada yele, Vilya, Villayadel*
Konkani	*Phodi paan*
English:	*Betle, Betle pepper, Betle-vine*
Persian	*Burg-e-Tanbol*
Arabic:	*Tanbol*
Chamorro:	*Papulu*
Thai:	*Plue, Pelu*

Sources: Dwivedi and Tripathi (2014) and Vikash *et al.* (2012)

Table 2.7 Taxonomic classification of *paan*

Kingdom	Plantae
Division	Magnoliphyta
Class	Magnolipsida
Order	Piperales
Family	Piperaceae
Genus	*Piper*
Species	*betle*

Source: Azahar *et al.* (2020)

2.3.1 Botanical description

The plant is a dioecious root climber, and the shoots reach any height from 3 to 10 m according to available facilities for climbing. The plant bears lateral branches along its entire length that grow a couple of feet from the ground. The stems are swollen and articulate, with dichotomous branching and rooting at the nodes. The stems are stout, almost terete, slightly flattened; when young, they are light green and marked by short, raised, whitish streaks and

with pinkish stripes along the node. The internodes generally attain a length of about 12 cm. and a diameter of 1.2 cm. Leaves are characterized as a simple blade, alternate, spiral and ex-stipulate; petioles are 2–5 mm long, pubescent and channeled. Leaf blades are glabrous, coriaceous, fleshy, greenish to yellowish, shining, broadly ovate, width 7–8.5 cm, length 9–11 cm; base cordate; apex acuminate; margin is entire, narrowly recurved; venation reticulate, 7–9 veins in two or three pairs coming from the midrib, one pair elevating from base. The inflorescence is an axillary spike up to 5.5 cm long. The male inflorescence forms a cylindrical pendulous catkin of 10 cm in length and 2 cm in diameter. Female spikes are also cylindrical, pendulous; length 2.5–4 cm and diameter 0.5 cm. Individual flowers are very minute and unisexual, reduced, consisting of a couple of stamens and stigmas inserted into the axil of each bract. The bracts are orbicular, peltate, arranged in a thickly crowded spiral series. The mature inflorescence is strongly aromatic. Fruiting spikes are 3–5 cm in length, orange and drupping, entrenched on the rachis of the mature inflorescence (Chibber, 1913; Chaveerach *et al.*, 2006)

2.3.2 Phytochemical profile

Piper betle is one of the extensively investigated plants for its various phytochemical constituents present in it, and the study revealed that the plant contains a wide range of phytochemicals that are biologically active. Compound concentrations depend on the different varieties of the plant, season, climate and may geographical location and also might be influenced by various factors such as soil, humidity, agronomic practices, rainfall, season and type of plant (Garg and Jain, 1996). The main phytochemical constituents of the essential oil of the betel leaf are mainly phenols and terpenes (Atal *et al.*, 1975). Table 2.8 lists the phytochemical components of *P. betle.* Since this dissertation is about the alcohol extraction of *P. betle*, emphasis has been placed on the chemical from *P. betle* leaves that was extracted using alcohol.

Table 2.8 Phytochemical components of *P. betle*

Plant part/Extract/Essential oil	Techniques of analysis	Chemical compounds
Aqueous extract of leaves	GC/MS	2,3-bis(hydroxy)propyl ester, 2-monopalmitin, α-hydroxy, alpha-hydroxyphenyl, benzeneacetic acid, benzeneacetic acid, hexadecanamide, hexadecanoic acid, hexadecanoic acid, hydroxychavicol, myristic acid, octadecanoic acid, octadecanoic acid
Essential oil from leaves	GC/MS	4-allyl-1,2-diacetoxybenzene, acetyleugenol, bicyclo(4.1.0)hept-3-en-camphene, chavicol, cis-ocimene, cyclohexene,4-methyl-decanal, eugenol, germacrene B, germacrene D, globulol, ledene, linalyl acetate, l-limonene, methyl-eugenol, phenyl acetylaldehyde, t-caryophyllene, t-ocimene, undecanal, α –humulene, α-pinene, β-elemene, β-myrcene, γ-cadinene, γ-ionene, γ-muurolene
Leaf extract	DART-MS	chavicol, allylpyrocatechol, chavibetol, phenyl alanine, chavicol acetate, allylpyrocatechol acetate, chavibetol acetate, allylpyrocatechol, diacetate
Volatile oil from leaves	GC/MS	β- caryophyllene, α-farnesene, α-humulene, germacrene b, germacrene d
Hexane extract of leaves	GC/MS	2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one, phellandrene, α-terpinene, p-cymene, sabinene, ɣ-terpinene, o-guaiacol, linalool, tujene, terpine-1-ol, terpine-4-ol, α-terpineol, safrole, eugenol, isoeugenol, α-copaene, β –bourbonene, methyleugenol, β –caryophyllene, β –cubebene

Table 2.8 continued…

Plant part/Extract/Essential oil	Techniques of analysis	Chemical compounds
Ethanol extract of leaves	GC/MS	heptafluorobutyrate, ethyl diazoacetate, 4-(2-propenyl) phenol, 3-fluoro-2-propynenitrite, eugenol, tris(trifluoromethyl)phosphine
Aqueous and ethanol extracts of leaves	GC/MS	*amino acid*: alanine, valine, isoleucine, proline *fatty acids*: palmitic acid, linoleic acid, linolenic acid, oleic acid, stearic acid, palmitic acid derivatives *sterols*: cholesterol, cholesterol derivatives, stigmasterol, β -sitosterol
Ethanol extract of leaves	GC/MS	1-phenylpropene-3,3-diol diacetate, eugenol, 4-chromanol, 4-allyl-1,2-diacetoxybenzene, hydroxychavicol (1-allyl-3, 4-dihydroxybenzene)

Sources: Bajpai *et al.* (2012), Bhat *et al.* (2015), Muruganandam *et al.* (2017), Nalina and Rahim (2007), Oliveira *et al.* (2016), Parthasarathy *et al.* (2014), WendyVoon *et al.* (2014) and Prakash *et al.* (2010).

2.3.3 Bioactive composition

Piper betle ability to act as a medium for any possible treatment practice in traditional medication is due to its many bioactive components. The bioactive components can be obtained from the extraction process performed on different parts of *Piper betle* such as leaves, vines, branch or roots. *Piper betle* major components consist of tannins, flavonoids (quercetin), eugenol, hydroxychavicol and chavibetol (Pradhan *et al.*, 2013; Dwivedi and Tripathi, 2014; Patil *et al.*, 2015).

2.3.4 Medicinal applications of *paan*

Leaves of *P. betle* L. (Family, Piperaceae) are commonly used as a masticator in Asia and as a traditional medicine in different countries. Its leaf extract has been reported to stimulate pancreatic lipase activity and to inhibit radiation-induced lipid peroxidation. The extract also increases the activities of antioxidants. Its hepatoprotective effect is well understood. Further, its methanolic leaf extract was found to exhibit immunomodulatory activity (Panda et al., 2018).

- Antidiabetic activity: *Piper betle* extract (hot water extract and cold ethanolic extract) possess marked hypoglycaemic activity and the findings suggested the ability of *Piper betle* in doing insulinomimetic activity (Azahar *et al.*, 2020).
- Antioxidant activity: Antioxidant activity of test substances was evaluated by the standard methods. The *P. betle* leaf extract showed antioxidant activity in NO and hydroxyl radical assays (Anjum *et al.*, 2020).
- Immunosuppressive activity: The study indicated the potential of methanolic extract of *P. betle* (MPb) as a novel candidate for immunosuppressive activity. The same could be further evaluated for its anticancer activity or as a potential candidate in the treatment of autoimmune disorders such as rheumatoid arthritis, systemic lupus erythomatous or emphysema (Kanjwani *et al.*, 2008).
- Anticancer activity: The leaf extract of *P. betle* exerts antioxidant activity, also inhibits the viability and migration of MCF-7 cells. Thus, the extract has promising potential for development into an anticancer agent for breast cancer f breast cancer (Supavadee *et al.*, 2019).
- Antifungal activity: The anticandidal activity of ethanolic and ethyl acetate extracts of *betel* and *tulsi* leaves along with standard drug fluconazole was analyzed (Basireddy *et al.*, 2019)
- Antibacterial activity: The antibacterial and antifungal properties and safety profiles of betel leaves firmly support their application in the development of various products, especially in the food and pharmaceutical industries (NiMade *et al.*, 2021)
- Food preservative: *P. betle* leaves have an antibiotic and antibacterial action that helps the extract preserve food (NiMade *et al.*, 2021). In the food industry, essential oil is a promising food additive to protect and enhance the shelf life of products during processing and storage (Prakash *et al.*, 2010).

Part III

Materials and methods

3.1 Materials

3.1.1 Raw materials and source

Molasses and sugar were purchased from the local market of Dharan. The three-year old, fresh stem of *T. cordifolia* was collected from the local village of Dharan in the month of July–September. The one and half-year old, fresh leaves of betel leaf were collected from the local village of Dharan in the month of July–September.

3.1.2 Equipment, glassware and chemicals

The following equipment, glassware, and equipment were used for the work and were available from the laboratory of Central Campus of Technology, Dharan.

3.1.2.1 Equipment and glassware

Fermentation jar (water jar), knives, hand refractometer (0-30, ATAGO, made in Japan), electrical balance (Phoenix instrument), pH meter (Labtronics, made in India), Spectrophotometer (hollow cathode and deuterium, made in Shimazadu Japan), burette and pipette (Kimble Glass, Inc., No. 17124-F), gas stoves, magnetic stirrer (Faithful, SH-4C) thermometer, mortar and pestle, Hot air oven, and daily routine glassware were used that were available in the campus.

3.1.2.2 Chemicals

The chemicals used during chemical analysis are listed as follows:

1. Ammonium phosphate or ammonium sulfate
2. Sulfuric acid (H_2SO_4)
3. 95% denatured alcohol
4. Yeast, *Saccharomyces cerevisiae* ADY (Safceno^{TM} SC22)
5. Methanol
6. Sodium carbonate
7. Potassium permanganate
8. Sodium thiosulphate

3.2 Methods

3.2.1 Preparation of rum

10 kg of sugarcane molasses was taken whose TSS was observed 81.83 °Bx. Then, the TSS was adjusted to 17° Bx. by adding distilled water of 35 L to make 45 L and the pH of solution is adjusted to 4.5 by adding conc.H_2SO_4. Then the solution was pasteurized to 85°C for 2 min to inhibit the growth of undesirable microorganisms and then allowed to cool at the room temperature, i.e., 25°C. After cooling, 0.5 g/L ADY and 0.5 g/L yeast nutrient were added and the juice was kept in three flasks of 20 L capacity for fermentation and loosely sealed with plastic. After the completion of fermentation, distillation was done to produce rum. The flowchart for rum production in this word is the same as shown in Fig. 2.1.

3.2.2 Herb powder

3.2.2.1 *Gurjo* powder

Gurjo or *Tinospora cordifolia* was cleaned with distilled water, then cut into small pieces about 3×3 mm^2. The small pieces were dried by conventional drying i.e. in cabinet/tray dryers at a temperature 45°C (Sarala *et al.*, 2012). After drying the dried *gurjo* was powdered by using mortar and pestle.

3.2.2.2 Betel leaves powder

Betel leaves were collected from the local village of Dharan. The leaves were cleaned with tissue paper to remove dirt. The initial moisture content for fresh leaf was determined as 84.97% WB using hot air oven method according to KC and Rai (2007). The leaves were dried by conventional drying ,i.e., in cabinet/tray dryers at a temperature 40°C to minimize loss of volatile components (Pin *et al.*, 2009a). After drying the dried Betel leaves were powdered using an electric grinder.

3.2.3 Extract of herbs

Herb extract were prepared according to the method followed by Satija *et al.* (2015) (Fig. 3.1).

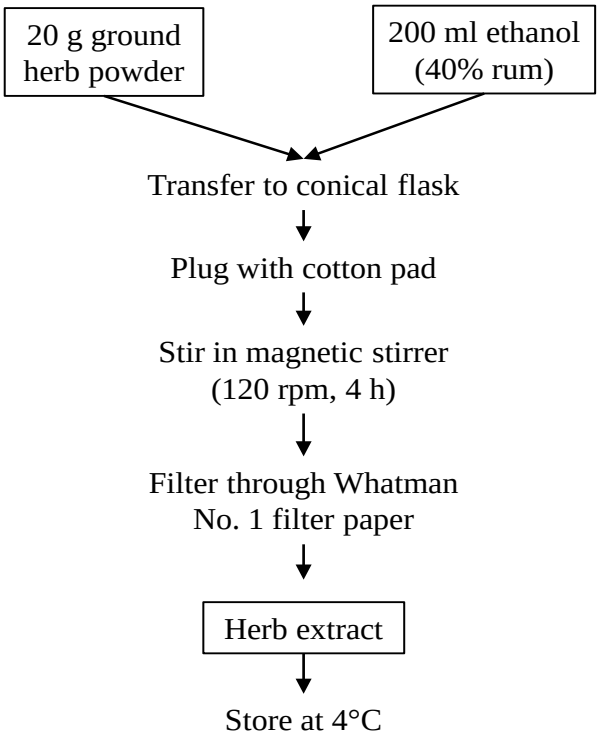

Fig. 3.1 Preparation of herb extract

Slightly modified Satija *et al.* (2015)

Thus, the concentration of herb extract concentrate can be determined as:

Amount of herb extracted in solvent = Wt. of dried herb taken for extraction – Wt. of dried residue after extraction.

$$\text{Concentration of herb extract concentrate} = \frac{\text{Wt. of herb extract in the solvent}}{\text{Final wt. of concentrated extract}}$$

3.2.4 Optimization of herb in rum

Afterwards, the prepared rum will be adjusted to 40% ethanol with the addition of distilled water. The herbal rum was optimized for two different herbs separately. The sensory threshold determination by trial method. Sensory evaluation was done in order to optimize the percentage of herbs in terms of color, flavor, taste, after taste and overall acceptance. Similarly, further optimization was done by varying the amount of herb (0.05-0.25%) for extract of *gurjo* and (0.3-0.15%) for extract of betel leaf along with chemical parameters (methanol, fusel oil, total polyphenol, antioxidant capacity) of treated sample were analyzed.

3.2.5 Criteria for optimization for herbal rum

Criteria for optimization of herbal rum are shown in Table 3.1 and Table 3.2.

Table 3.1 Sensory parameters

Parameters	Goals
Appearance	In range
Taste	Maximize
Flavor	Maximize
After Taste	Maximize
Overall acceptance	Maximize

Table 3.2 Chemical parameters

Parameters	Goals
Fusel oil	Maximize*
Methanol	Minimize*
Total polyphenol	Health beneficial range
Antioxidant	Health beneficial range

* denotes in range according to the international standard.

3.2.6 Working plan

The experimental plan for preparation of *gurjo* extract blended rum and plan of betel leaf extract blended rum are shown in Fig. 3.2 and Fig 3.3.

3.3 Analytical parameters of distillate/ herbal rum

3.3.1 Chemical analysis

3.3.1.1 Determination of antioxidant

Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by Blois (1958). A 0.1 mM stock solution of DPPH was prepared, which was

further diluted to adjust the absorbance value of approximately 0.9 at the wavelength of λ=517 nm. The absorbance of DPPH radical solution (A_0, control) was measured by adding 2.5 ml working DPPH solution to 0.5 ml of 80% methanol.

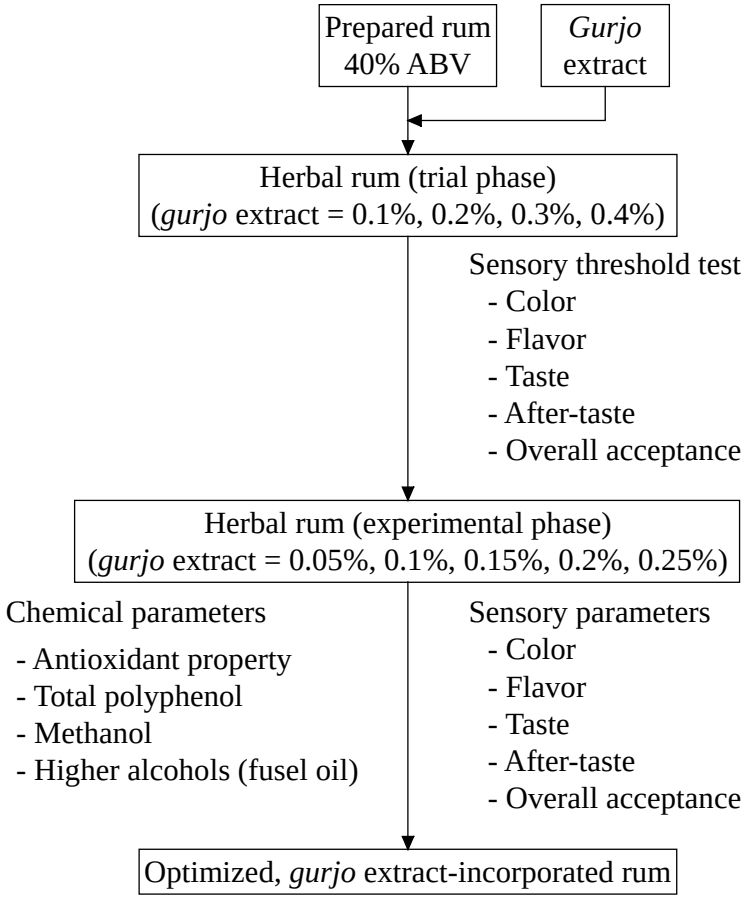

Fig. 3.2 Flow sheet for experimental plan of *gurjo* extract blended rum

Three different concentrations of methanolic solutions of each extract were prepared by the serial dilution of the stock solution (10 mg/ml) of the respective extract. To each 0.5 ml extract solution (as Sample), 2.5 ml of 0.1 mM DPPH solution was added. These samples were shaken well and kept in dark for 30 min at room temperature. The absorbance was measured at 517 nm against the control solution. The radical scavenging activity was expressed as the radical scavenging percentage using the following equation:

Inhibition % = 100 (A_0-As)/As, where:

A_0 = average absorbance of the control (DPPH) solution

As = average absorbance of the sample

3.3.1.2 Determination of total polyphenol

The total phenolic contents of the ethanolic extracts of herb were estimated using the Folin-Ciocalteau reagent as described by Siddiqui *et al.* (2017)

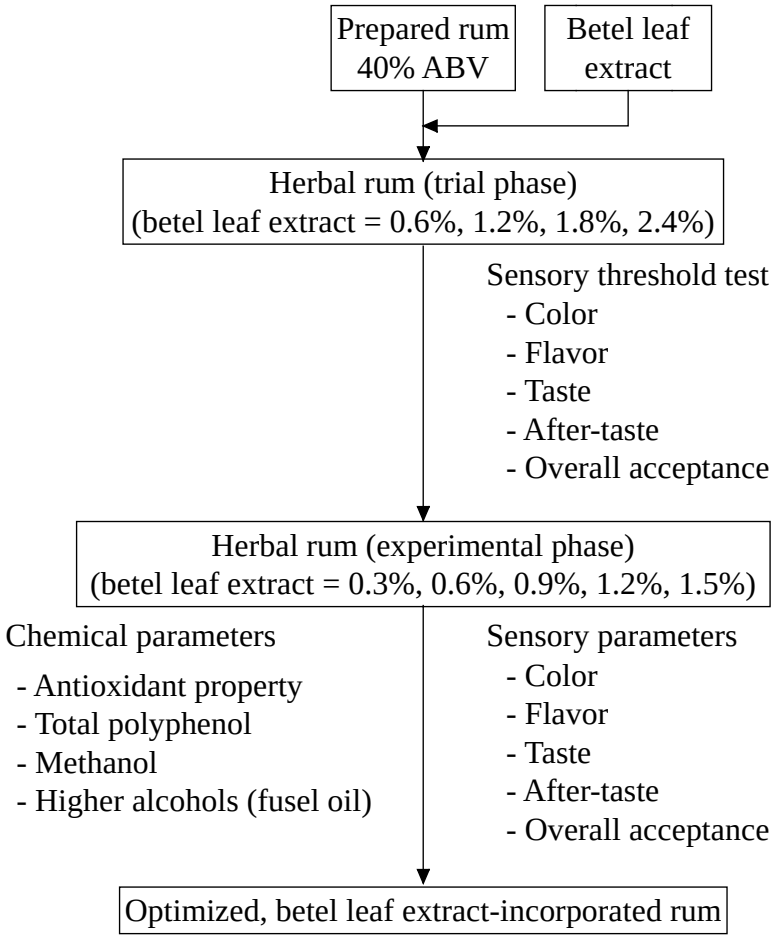

Fig. 3.3 Flow sheet for experimental plan of betel leaf extract blended rum

3.3.1.3 Determination of methanol content in spirit

Methanol content was determined by chromotropic acid colorimetric method as per AOAC (2005).

3.3.1.4 Determination of higher alcohol (fusel oil) in spirit.

Fusel oil content was determined by spectrophotometric method as per AOAC Official method (AOAC, 2005).

3.3.2 Sensory analysis

The sensory analysis of the product in terms of taste, flavor, appearance, and overall acceptability was carried out using 9-point hedonic rating (like extremely = 9, dislike extremely = 1). The sensory evaluation for the overall quality was carried out with 16 semi-trained panelists of CCT, Dharan which included the faculties and students of age group 22-55.

3.4 Data analysis

The data obtained during the course of experiment will be first processed and then analyzed by Microsoft office professional Plus 2016. The mean values were compared by using LSD method Genstat twelfth edition, version-12.1.0.3338. Means of the data were separated whether they are significant or not by using Tukey method at 5% level of significance.

Part IV

Results and discussion

The molasses and sugar were brought from the local market and the molasses were diluted to describe level of TSS (17 °Bx) and pH (4.5) and inoculated with culture (active dry yeast). Fermentation was carried out at room temperature 29±4°C.

4.1 Physiochemical properties of raw molasses

The physiochemical properties of raw molasses are given in Table 4.1

Table 4.1 physiochemical properties of raw molasses.

Parameters	Values
TSS	81.83°Bx
pH	5.63

Total soluble solids and pH were analyzed, and the obtained values are tabulated in Table 4.1. The values are similar to those described in (Carioca, 2011).

4.2 The moisture content of herbs

The percentage of moisture on wet basis of the herbs are given in Table 4.2.

Table 4.2 Moisture content in herbs.

Fresh Herb	% Moisture in (WB)
Gurjo	34.39%
Betel leaves	84.97%

The moisture content is found in stem of g*urjo* is described by Mahima *et al.* (2014) and moisture content of betel leaves is described by Pin *et al.* (2009a).

4.3 The alcohol content of rum

The rum had 54% v/v ethanol content which was adjusted to 40%. The rum had a highly characteristic bouquet.

According to Rai and Subba (2016) single-column distillate, assumed to contain above 55% abv can be blended with distilled water to give rum of 40% abv.

4.4 Sensory evaluation

4.4.1 Sensory evaluation of *gurjo* blended rum for threshold determination

The raw data of the sensory evaluation and the ANOVA are tabulated in Appendix B. Semi-trained panelist carried out sensory evaluation of all five formulations based on appearance, flavor, taste, after taste and overall acceptability. The obtained data from sensory evaluation was analyzed using two-way ANOVA at 5% significance level to study the significant difference among the formulations made and among panelists. There was no significant difference among formulations made and among panelists. There was no significant difference in all panelists judging the specified parameters of the products. Mean scores of the products in all parameters as obtained from sensory analysis is given in Fig. 4.1, A, B, C, and D denotes 0.1%, 0.2%, 0.3% and 0.4% of *gurjo* extract blended rum, respectively.

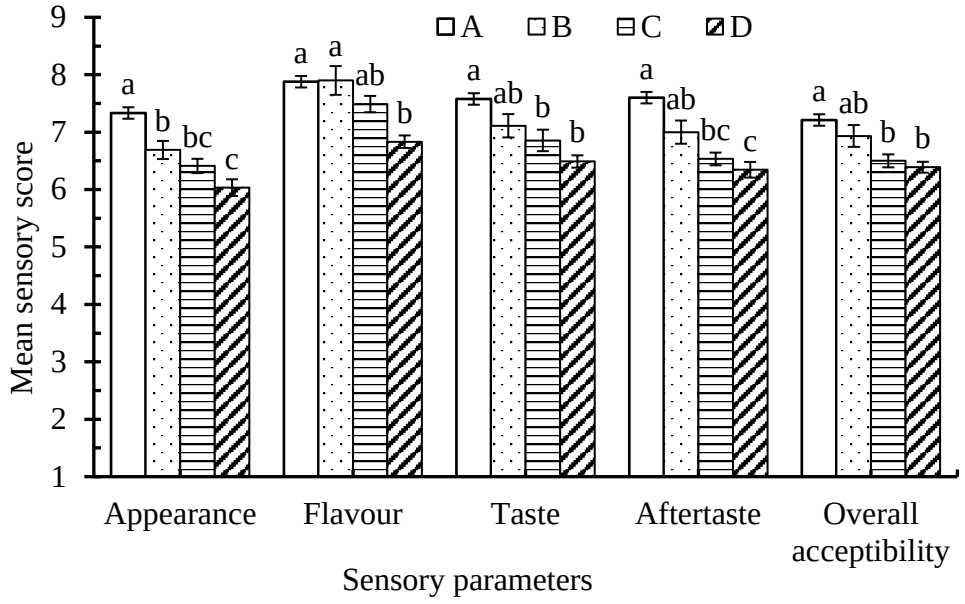

Fig. 4.1 Effect of *gurjo* extract variation on sensory threshold determination

4.4.1.1 Effect on appearance

The mean sensory score for the appearance of the sample A, B, C, D were found to be 7.3, 6.6, 6.4 and 6.0 respectively as shown in Fig. 4.1. The mean score was found to be highest for sample A (7.3). Samples A, B and D were found to be significantly different while sample C was found to be not significant different to sample B and D respectively at 5% level of significant in the statistical analysis represent the mean sensory score for color of *gurjo* extract blended rum. Vertical error bars represent standard deviation of score given by the panelists.

4.4.1.2 Effect on flavor

Fig 4.1 represents the mean sensory scores for flavor of herbal rum. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of score given by panelists. The mean sensory score for the flavor of the sample A, B, C and D were found to be 7.8, 7.9, 7.4 and 6.8 respectively. The mean score was found to be highest for sample B (7.9). Sample A, B and C are not significant different at 5% level of significant whereas Sample A and D have significant different at 5% level of significant.

4.4.1.3 Effect on taste

Fig 4.1 represents the mean sensory score for taste of *gurjo* extract blended rum Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of score given by panelists.

The mean sensory score for the taste of the samples A, B, C and D were found to be 7.5, 7.1, 6.8 and 6.4 respectively as shown in Fig. 4.1. The mean score was found to be highest for sample A (7.5). Sample C and D were not found to be significantly different while others are found to be significantly different at 5% level of significance.

4.4.1.4 Effect on after taste

Fig 4.1 represents the mean sensory score for after taste of *gurjo* extract blended rum. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of score given by panelists.

The mean sensory score for the after taste of the samples A, B, C and D were found to be 7.6, 7.0, 6.5 and 6.3 respectively as shown in Fig. 4.1. The mean score was found to be highest for sample A (7.6). Sample A, B, C and D were found to be significantly different at 5% level of significance.

4.4.1.5 Effect on overall acceptance

Fig 4.1 represents the mean sensory score for taste of *gurjo* extract blended rum. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of score given by panelists.

The mean sensory score for the overall acceptance of the samples A, B, C and D were found to be 7.2, 6.9, 6.5 and 6.3 respectively as shown in Fig. 4.1. The mean score was found to be highest for sample A (7.2). Sample B, C and D were found to be not significantly different while others are found to be significantly different at 5% level of significance.

4.4.1.6 Sensory responses for optimization

From the score obtained through sensory evaluation, rum made from formulation having up to 0.2% *gurjo* extract blended rum seems to be the superior product in terms of sensory parameters. From the sensory evaluation products having more than 0.25% *gurjo* extract blended rum are undesirable in terms of appearance, flavor, taste, aftertaste and overall acceptance. Therefore, products containing up to 0.25% *gurjo* extract blended rum are further optimized.

4.4.2 Sensory evaluation of *gurjo* extract blended rum

The raw data of the sensory evaluation and the ANOVA are tabulated in Appendix B. The summary of the statistical test for difference between the treatment for varying percentage of *gurjo* extract in rum to optimize the amount of *gurjo* extract. Semi trained panelists carried out sensory evaluation of all four formulations based on appearance, flavor, taste, aftertaste and overall acceptability as in threshold determination. Products having 0.05%, 0.10%, 0.15%, 0.20% and 0.25% *gurjo* extract were denoted by M, N, O, P and Q respectively.

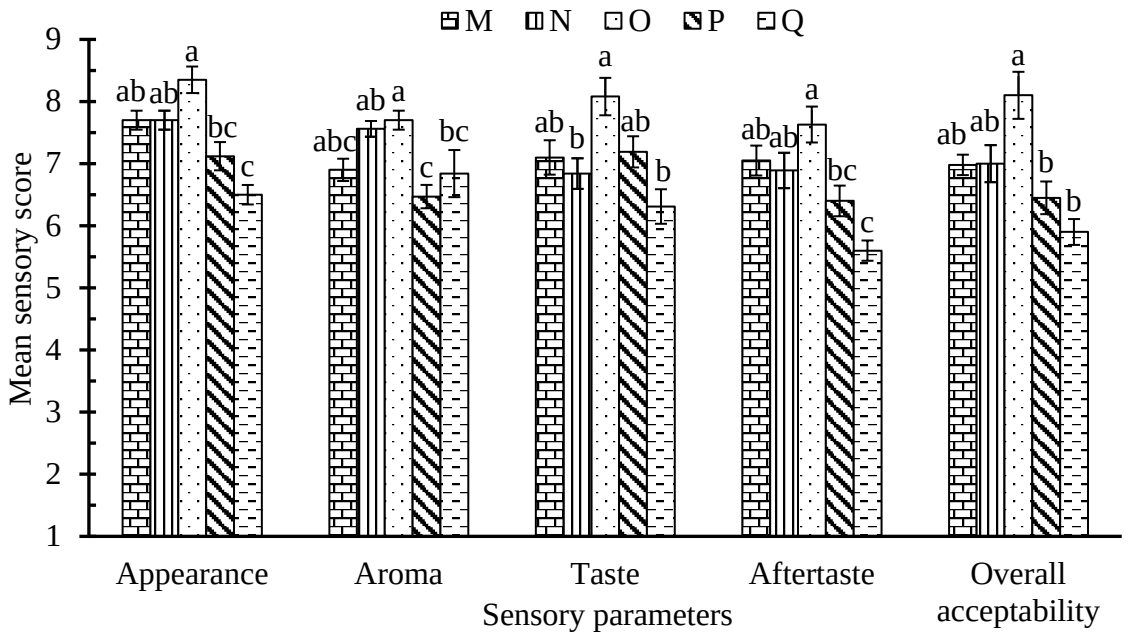

Fig. 4.2 Effect of *gurjo* extract variation on sensory evaluation

Fig 4.2 represents the mean sensory of *gurjo* extract blended rum. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by panelist.

4.4.2.1 Effect on appearance

The mean sensory score for the appearance of the sample M, N, O, P, and Q were found to be 7.7, 7.7, 8.3, 7.12 and 6.5 respectively as shown in Fig. 4.2. The mean score was found to be highest for sample O (8.3). Sample M, N & M, O were found to be significantly indifference and sample O, N; O, P; O, Q; M, P; M, Q; N, P; N, Q and P, Q were found to be significantly difference in appearance at 5% level of significance in the statistical analysis.

Stem of the *gurjo* appears, young stems are green while the older ones show a light brown (Upadhyay *et al.*, 2010). So, the extract of *gurjo* is green brownish. Herb or spice can be enriched in rum during the mixing phase and is usually dark in color (Mangwanda *et al.*, 2021). Sample M, N were least score by panelist since the appearance is weak greenish, similarly formulation P, Q were also least score, as man eats with his eyes.

4.4.2.2 Effect on flavor

The mean sensory score for the appearance of the sample M, N, O, P, and Q were found to be 6.9, 7.5, 7.7, 6.4 and 6.8 respectively as shown in Fig. 4.2. The mean score was found to be highest for sample O (7.7). Sample M, N; M, O; N, O and P, Q were found to be significantly indifference and sample M, P; M, Q; N, P; N, Q; O, P and O, Q were found to be significantly difference in flavor at 5% level of significance in the statistical analysis.

Since the aroma of rum is very unique (Mangwanda *et al.*, 2021). Herbal extract has slightly unpleasant, specific bitter taste (Berketova *et al.*, 2020), This may slightly lower the aroma capacity. In comparison to other formulations, the formulation O having 0.15% *gurjo* extract blended rum had an acceptable aroma.

4.4.2.3 Effect on Taste

The mean sensory score for the appearance of the sample M, N, O, P, and Q were found to be 7.1, 6.8, 8.0, 7.1 and 6.3 respectively as shown in Fig. 4.2. The mean score was found to be highest for sample O (8.0). Sample M, N; M, O; M, P; M, Q; N, Q and O, P were found to be significantly indifference and sample N, O; N, P; O, Q and P, Q were found to be significantly difference in taste at 5% level of significance in the statistical analysis.

The taste of *gurjo* is bitter and bitter tastes are responsible for bitter acids. These range from alkaloids, like quinine, to polyphenols, like tannins. Tannins are a good indication of bitterness in foods (Upadhyaya *et al.*, 2011). This is neutralized by rum at a certain limit. Among all formulations, formulation O had the best acceptable taste as chosen by the panelists.

4.4.2.4 Effect on Aftertaste

The mean sensory score for the appearance of the sample M, N, O, P, and Q were found to be 7.0, 6.8, 8.0, 7.1 and 6.3 respectively as shown in Fig. 4.2. The mean score was found to be highest for sample O (8.0). Sample M, N; M, O; N, O and P, Q were found to be significantly indifference and sample M, P; M, Q; N, P; N, Q; O, P and O, Q were found to be significantly difference in aftertaste at 5% level of significance in the statistical analysis.

The aftertaste enhanced the taste after drinking. The aftertaste may be bitter, sweet, spicy, etc. (Ickes and Cadwallader, 2018). In the present study, the aftertaste was bitter-sweet, which may be due to a combination of herbs and alcohol.

4.4.2.5 Effect on Overall acceptance

The mean sensory score for the appearance of the sample M, N, O, P, and Q were found to be 6.9, 7.0, 8.1, 6.4 and 5.9 respectively as shown in Fig. 4.2. The mean score was found to be highest for sample O (8.1). Sample O, P and O, Q were found to be significantly difference and sample M, N; M, O; M, P; M, Q; N, O; N, P; N, Q and P, Q were found to be significantly indifference in overall acceptance at 5% level of significance in the statistical analysis.

Overall acceptance is determined by overall attributes, which are scored in terms of likes and dislikes. In the present study, formulation O was the best product.

4.4.3 Sensory evaluation of betel leaf extract blended rum for threshold determination

The raw data of the sensory evaluation and the ANOVA are tabulated in Appendix B, respectively. Semi-trained panelist carried out sensory evaluation of all five formulations based on appearance, flavor, taste, after taste and overall acceptability. The obtained data from sensory evaluation was analyzed using two-way ANOVA at 5% significance level to study the significant difference among formulation made and among panelists. There was no significant difference among formulations made and among panelists. There was no significant difference in all panelists judging the specified parameters of the products. Mean scores of the products in all parameters as obtained from sensory analysis are shown in Fig. 4.3. A, B, C, and D denote 0.6%, 1.2%, 1.8% and 2.4%, respectively.

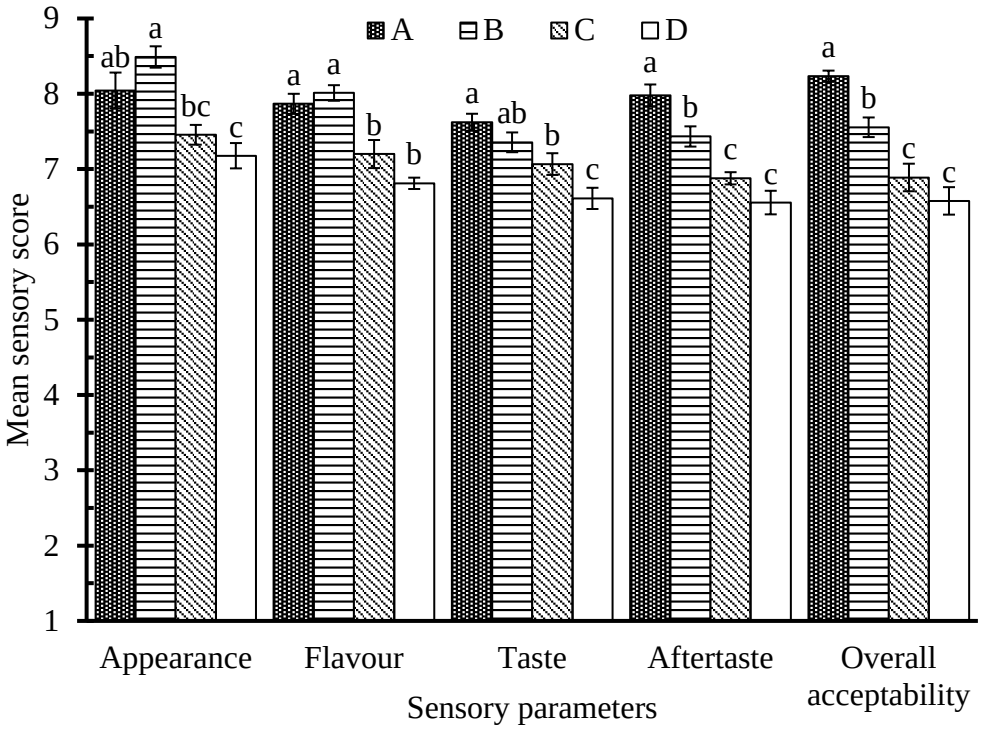

Fig. 4.3 Effect of betel leaf extract variation on sensory threshold determination.

4.4.3.1 Effect on appearance

The mean sensory score for the appearance of sample A, B, C, D were found to be 8.0, 8.4, 7.4 and 7.1 respectively as shown in Fig. 4.3. The mean score was found to be highest for sample B (8.4). Samples A and B and C and D were found to be significantly indifferent at 5% level of significant in the statistical analysis represent the mean sensory score for appearance of betel leaf extract incorporate rum. Vertical error bars represent standard deviation of score given by the panelists.

4.4.3.2 Effect on flavor

Fig 4.3 represents the mean sensory scores for flavor of betel-leaf extract blended rum. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of score given by panelists. The mean sensory scores for the flavor of sample A, B, C and D were found to be 7.8, 8.0, 7.2 and 6.8 respectively. The mean score was found to be highest for sample B (8.0). Sample A and B, and C and D were found significant indifferent at 5% level of significant.

4.4.3.3 Effect on taste

Fig 4.3 represents the mean sensory score for taste of betel-leaf extract blended rum. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of score given by panelists.

The mean sensory score for the taste of the samples A, B, C and D were found to be 7.6, 7.3, 7.0 and 6.6 respectively as shown in Fig. 4.3. The mean score was found to be highest for sample A (7.6). Sample A and B were found to be significantly indifferent at 5% level of significance.

4.4.3.4 Effect on after taste

Fig 4.3 represents the mean sensory score for aftertaste of betel-leaf extract blended rum. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of score given by panelists.

The mean sensory score for the after taste of the samples A, B, C and D were found to be 7.9, 7.4, 6.8 and 6.5 respectively as shown in Fig. 4.3. The mean score was found to be highest for sample A (7.9). Sample C and D were found to be significantly indifferent at 5% level of significance.

4.4.1.5 Effect on overall acceptance

Fig 4.3 represents the mean sensory score for overall acceptance of betel-leaf extract blended rum. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of score given by panelists.

The mean sensory score for the overall acceptance of samples A, B, C and D were found to be 8.2, 7.5, 6.8 and 6.5 respectively as shown in Fig. 4.3. The mean score was found to be highest for sample A (8.2). Sample C and D were found to be significantly indifferent at 5% level of significance.

4.4.1.6 Sensory responses for optimization of betel-leaf extract blended rum

From the score obtained through sensory evaluation, rum made from formulation having more than 1.2% betel-leaf extract blended rum seems to be the worse product in terms of sensory parameters. From the sensory evaluation products having less than 1.5% betel-leaf extract blended rum are desirable in terms of appearance, flavor, taste, aftertaste and overall acceptance. Therefore, products containing up to 1.5 % betel-leaf extract blended rum were further optimized.

4.4.4 Sensory evaluation of betel-leaf extract blended rum

The raw data of the sensory evaluation and the ANOVA are tabulated in Appendix B. Semi-trained panelists carried out sensory evaluation of all five formulations based on appearance, flavor, taste, aftertaste and overall acceptability as in threshold determination. Products having 0.3%, 0.6%, 0.9%, 1.2% and 1.5% betel-leaf extract were denoted by M', N', O', P' and Q' respectively.

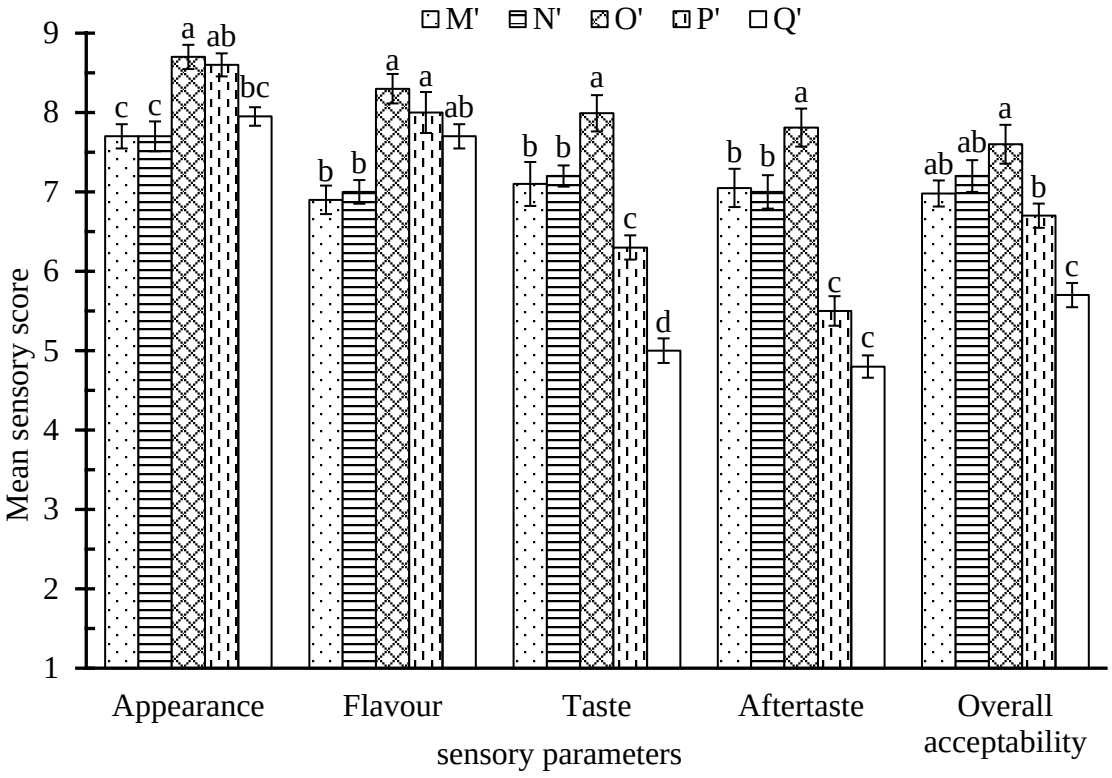

Fig. 4.4 Effect of betel leaf extract variation on sensory evaluation

Fig 4.4 represents the mean sensory of betel-leaf extract blended rum. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by panelist.

4.4.4.1 Effect on appearance

The mean sensory score for the appearance of the sample M', N', O', P', and Q' were found to be 7.7, 7.7, 8.7, 8.6 and 7.9 respectively as shown in Fig. 4.4. The mean score was found to be highest for sample O' (8.7). Sample M', N'; M', Q'; N', Q' and O', P' were found to be significantly indifference and sample M', O'; M', P'; N', O'; N', P' and O', P' were found to be significantly difference in appearance at 5% level of significance in the statistical analysis.

The present study analyzed the effect of different concentration of betel leaves extracts on the appearance of rum. Ethanolic extraction is used to extract many essential oils and other compounds. Aromatic essential oils like 4-allyl-1,2-diacetoxybenzene, acetyleugenol, bicyclo (4.1.0) hept-3-en- camphene, chavicol, cis-ocimene, cyclohexene,4-methyl-decanal, etc. are responsible for the yellowish color in the extract (WendyVoon *et al.*, 2014; NiMade *et al.*, 2021). The yellowish color of extract appears in rum as a light-yellow color. The sensory panelists gave formulation O' high marks.

4.4.4.2 Effect on flavor

The mean sensory score for the appearance of the sample M', N', O', P', and Q' were found to be 6.9, 7.0, 8.3, 8.0 and 7.7 respectively as shown in Fig. 4.2. The mean score was found to be highest for sample O' (8.3). Sample M', N'; M', Q'; N', Q'; O', P'; O', Q' and P', Q' were found to be significantly indifference and sample M', O'; M', P'; N', O' and N', P' were found to be significantly difference in flavor at 5% level of significance in the statistical analysis.

Since the aroma of rum is very unique (Mangwanda *et al.*, 2021). Betel leaves extract has slightly unpleasant, burning test due to volatile aromatic compound (WendyVoon *et al.*, 2014; NiMade *et al.*, 2021). This may slightly lower the aroma capacity. In comparison to other formulations, the formulation O' betel leaf extract blended rum had an acceptable aroma.

4.4.4.3 Effect on taste

The mean sensory score for the appearance of the sample M', N', O', P', and Q' were found to be 7.1, 7.2, 7.9, 6.3 and 5.0 respectively as shown in Fig. 4.2. The mean score was found to be highest for sample O' (7.9). Sample M', N' and N', O' were found to be significantly indifference and sample M', O'; M', P'; M', Q'; N', P'; N', Q'; O', P'; O', Q' and P', Q' were found to be significantly difference in taste at 5% level of significance in the statistical analysis.

The taste of betel leaf extract is burnt taste. This burnt taste is responsible for aromatic compounds (WendyVoon *et al.*, 2014). This is neutralized by rum at a certain limit. Among all formulations, formulation O' had the most acceptable taste as chosen by the panelists.

4.4.4.4 Effect on aftertaste

The mean sensory score for the appearance of the sample M', N', O', P', and Q' were found to be 7.0, 7.0, 7.8, 5.5 and 4.8 respectively as shown in Fig. 4.4. The mean score was found to be highest for sample O' (7.8). Sample M', N'; M', O'; N', O' and P', Q' were found to be significantly indifference and sample M', P'; M', Q'; N', P'; N', Q'; O', P' and O', Q' were found to be significantly difference in aftertaste at 5% level of significance in the statistical analysis.

The aftertaste enhanced the taste after drinking. The aftertaste may be bitter, sweet, spicy, etc. (Ickes and Cadwallader, 2018). In our study, the aftertaste was burnt-sweet; this may be a combination of herbs and alcohol.

4.4.4.5 Effect on overall acceptance

The mean sensory score for the appearance of the sample M', N', O', P', and Q' were found to be 6.9, 7.2, 7.6, 6.7 and 5.7 respectively as shown in Fig. 4.2. The mean score was found to be highest for sample O' (7.6). Sample M', O'; M', P'; N', O' and N', P' were found to be significantly indifference and sample M', N'; M', Q'; N', Q'; O', P'; O', Q' and P', Q' were found to be significantly difference in overall acceptance at 5% level of significance in the statistical analysis.

Overall acceptance is determined by overall attributes, which are scored in terms of likes and dislikes. In our study, formulation O' is the best product.

4.5 Chemical analysis of *gurjo* incorporated rum

The chemical analysis of distillate and herbal rum were performed and the mean values of triplicated analysis are presented in Table 4.3

Table 4.3 Chemical composition of distillate and *gurjo* extract incorporated rum

Sample	Methanol (g/100 L)	Fusel oil (g/100 L)	Total polyphenol (g/100 L)	Antioxidant activity (% inhibition)
D_I	$37.2^{a} \pm 0.2$	$150.5^{a} \pm 1.80$	-	-
M	$36.1^{ab} \pm 1.15$	$148.2^{ab} \pm 1.89$	$11.22^{c} \pm 0.10$	$72.26^{bc} \pm 1.18$
N	$34.74^{bc} \pm 1.08$	$144.8^{bc} \pm 0.76$	$12.28^{bc} \pm 0.51$	$70.75^{c} \pm 0.50$
O	$33.52^{c} \pm 1.80$	$143.5^{c} \pm 1.32$	$12.07^{C} \pm 0.57$	$73.27^{ab} \pm 0.48$
P	$33.59^{c} \pm 1.80$	$142.5^{c} \pm 0.5$	$13.52^{ab} \pm 0.48$	$72.89^{ab} \pm 0.78$
Q	$30.47^{d} \pm 1.80$	$147.5^{ab} \pm 0.5$	$13.89^{a} \pm 0.51$	$74.71^{a} \pm 0.68$

D_I = 40% concentrate distilled rum, M = 0.05% *gurjo* extract incorporated rum, N = 0.10% *gurjo* extract incorporated rum, O = 0.15% *gurjo* extract incorporated rum, P = 0.20% *gurjo* extract incorporated rum, Q = 0.25% *gurjo* extract incorporated rum.

4.5.1 Fusel oil content

The average fusel oil content obtained from distilled from distilled sprit and *gurjo* extract incorporated rum (Di, M, N, O, P, Q) was found 150.5 g/100 L, 148.2 g/100 L, 144.8 g/100 L, 143.5 g/100 L, 142.5 g/100 L, and 147.5 g/100 L respectively. From the quantitative point of view, the formation of higher alcohols depends mainly on the yeast strain used. Due to the characteristic aroma, higher alcohols have a strong influence on the taste of spirits. Higher alcohols, with three to five carbons, have characteristic odors, traditionally associated with spirits. Above five carbons, these alcohols become oily, and some of them recall the

smell of flowers. The higher alcohols which correspond to sum of then- propyl, iso-butyl, iso-amyl, sec- butyl and n-butyl alcohols. Alcohols with more than two carbon atoms are generally known as higher alcohols (fusel alcohol). They are synthesized by yeast metabolism from amino acids, and are naturally present in all alcoholic beverages of agricultural origin. Their concentrations in sugarcane spirits are influenced by factors such as the care taken when cutting the cane, soil composition, time delay before grinding, yeast strain, and the temperature and aeration during fermentation (Yokota and Fagerson, 1971). The control sugarcane sprits used in the present study presented fusil oil content below the maximum limit given by international legislation of 200 g/100 L anhydrous alcohol where the concentration remains in spiced rum is almost near to distillate which may be due to the very little effect of spice on fusil oil content. These results can be compared with those obtained by (Ricardo, 2011) who produced three different sugarcane spirits with higher alcohol concentrations varying from 680.66 to 309.86 mg (100 ml anhydrous alcohols). The calibration curve for fusel oil content is shown in Appendix C.

4.5.2 Methanol content

The average methanol content obtained from distilled spirit and spiced rum M, N, O, P, Q was found to be 37.2 g/100 L, 36.1 g/100 L, 34.74 g/100 L, 33.52 g/100 L and 30.47 g/100 L respectively. The methanol concentrations in the rums were all below 50 g/100 L the concentration demanded by the East Africa legislation (EAC, 2001). Methanol is one of the volatile compounds that have attracted particular attention owing to its toxicity. It is, in fact, a neuro-toxic substance for humans, affecting the retina in particular, and various situations of intoxication by methanol have been detected associated with the consumption of adulterated alcoholic beverages with elevated contents of this alcohols. The origin of the methanol is connected to pectin degradation, and inadequate filtration of the must, allowing the presence of sugarcane pith in the fermentative process, although they did not differ from each other, but since it is partially soluble in water, the distillation of methanol starts in the head fraction and is exhausted at the start of the heart fraction (Paine and Dayan, 2001).

Similar concentration of methanol ranging from 10 g/100 L to 75 g/100 L were obtained by Pontes *et al.* (2006).

4.5.3 Total polyphenol content

The total phenolic content of the ethanolic extracts were calculated by using calibration curve shown in Appendix D obtained from the Gallic acid solutions ranging from 40 μg/ml to 70 μg/ml. Based on the equation obtained, total phenolic content was calculated and expressed in mg GAE/g of dried extract. The phenolic content is shown in Table 4.3. The total phenolic content of *gurjo* extract blended rum of sample M, N, O, P and Q were 11.22±0.10, 12.28±0.51, 12.07±0.57, 13.52±0.48 and 13.89±0.51 mg GAE/L. The total phenolic content of *gurjo* extract blended rum was found 11.22±0.10 mg GAE/L for sample M was maximum and 13.89±0.51 mg GAE/L for sample Q was minimum. Generally, the TPC is increases with respect to increase in concentration of *gurjo* extract. Similar concentration of total polyphenol content were obtained (Ganie *et al.*, 2013).

4.5.4 Antioxidant activity

The average DPPH activity (% inhibition) of *gurjo* extract incorporated rum (M, N, O, P, Q) was found to be 72.26, 70.75, 73.27, 72.89 and 74.74, respectively. In *Tinospora cordifolia* higher activity is shown even in lower concentration of extract although it depends upon in which solvent extract is extracted, DPPH Scavenging activity (%) of ethanol extraction is higher than that of methanol extraction of *gurjo* (Ganie *et al.*, 2013).

4.5.5 Optimization study

As regards the sensory scores and chemicals analysis, the optimization study is carried out as given in Table 4.4.

Table 4.4 Optimization goals for treatment (sensory)

Parameters	Optimized treatment
Color	O
Flavor	O
Taste	O
After taste	O
Overall acceptance	O

Regarding the sensory parameters, treatment O, i.e., 0.15% was found to be superior *gurjo* extract blended rum.

The optimization goals for chemical treatment are shown in Table 4.5.

Table 4.5 Optimized goals for treatment (chemical analysis)

Parameters	Optimized treatment	Remarks
Methanol (g/100 L)	O (33.52)	In the range
Fusel oil (g/100 L)	O (143.5)	In the range
Total polyphenol (mg GAE/L)	O (12.07)	In the rage of health benefit
Antioxidant (% inhabitation)	O (73.27)	In the rage of health benefit

From the study of different parameters, treatment O treated with 0.15% *gurjo* extract incorporate rum had superior sensory characteristics. Similarly, methanol and fusil oil are in the range and antioxidant and total polyphenol are in the health benefit range.

4.6 Chemical analysis of betel leaf extract incorporate rum

The chemical analyses of distillate and herbal rum were performed and the mean values of triplicated analyses are presented in Table 4.6.

Table 4.6 Chemical composition of distillate and betel leaf extract incorporated rum

Sample	Methanol (g/100 L)	Fusel oil (g/100 L)	Total polyphenol (g/100 L)	Antioxidant (% inhabitation)
D_I	37.2^{a} 0.2	150.5^{a} ±1.8	-	-
M'	36.8^{ab} ±0.3	148.2^{a} ±0.76	67.28^{c}±1.07	58.50^{c}±1.70
N'	35.33^{c} ±0.65	143.3^{bc} ±0.57	65.12^{c}±1.41	65.09^{b}±0.94
O'	36.83^{ab} ±0.30	144.5^{b} ±0.4	79.63^{b}±0.93	67.92^{ab}±0.94
P'	35.57^{bc} ±0.58	142.1^{cd} ±0.17	85.19^{a}±3.70	66.67^{b}±0.29
Q'	32.1^{d} ±0.85	140.8^{d} ±0.25	89.51^{a}±0.53	71.13^{a}±1.80

D_I = 40% concentrate distillated rum, M' = 0.3% betel leaf extract incorporated rum, N' = 0.6% betel leaf extract incorporated rum, O' = 0.9% betel leaf extract incorporated rum, P' = 1.20 betel leaf extract incorporated rum, Q' = 1.5% betel leaf extract incorporated rum.

4.6.1 Fusel oil content

The average fusel oil content obtained from distilled from distilled sprit and betel leaf extract incorporated rum (Di, M', N', O', P', Q') was found 150.5 g/100 L, 148.2 g/100 L, 143.3 g/100 L, 144.5 g/100 L, 142.1 g/100 L, and 140.8 g/100 L respectively. From the quantitative point of view, the formation of higher alcohols depends mainly on the yeast strain used. Due to the characteristic aroma, higher alcohols have a strong influence on the taste of spirits. Higher alcohols, with three to five carbons, have characteristic odors, traditionally associated with spirits. Above five carbons, these alcohols become oily, and some of them recall the smell of flowers. The higher alcohols which correspond to sum of then- propyl, iso-butyl, iso-amyl, sec- butyl and n-butyl alcohols. Alcohols with more than two carbon atoms are generally known as higher alcohols (fusel alcohol). They are synthesized by yeast metabolism from amino acids, and are naturally present in all alcoholic beverages of agricultural origin. Their concentrations in sugarcane spirits are influenced by factors such

as the care taken when cutting the cane, soil composition, time delay before grinding, yeast strain, and the temperature and aeration during fermentation (Yokota and Fagerson, 1971). The control sugarcane sprits used in the present study presented fusil oil content below the maximum limit given by international legislation of 200 g/100 L anhydrous alcohol where the concentration remains in spiced rum is almost near to distillate which may be due to the very little effect of spice on fusil oil content. These results can be compared with those obtained by (Ricardo, 2011) who produced three different sugarcane spirits with higher alcohol concentrations varying from 680.66 to 309.86 mg (100 ml anhydrous alcohols). The calibration curve for fusel oil content is shown in Appendix C.

4.6.2 Methanol content

The average methanol content obtained from distilled spirit and spiced rum (Di, M', N', O', P', Q') were found to be 37.2 g/100 L, 36.8 g/100 L, 35.33 g/100 L, 36.83 g/100 L, 35.57 g/100 L and 32.1 g/100 L respectively. The methanol concentrations in the rums were all below 50 g/100 L the concentration demanded by the East Africa legislation (EAC, 2001). Methanol is one of the volatile compounds that have attracted particular attention owing to its toxicity. It is, in fact, a neuro-toxic substance for humans, affecting the retina in particular, and various situations of intoxication by methanol have been detected associated with the consumption of adulterated alcoholic beverages with elevated contents of this alcohols. The origin of the methanol is connected to pectin degradation, and inadequate filtration of the must, allowing the presence of sugarcane pith in the fermentative process, although they did not differ from each other, but since it is partially soluble in water, the distillation of methanol starts in the head fraction and is exhausted at the start of the heart fraction (Paine and Dayan, 2001).

Similar concentration of methanol ranging from 10 g/100 L to 75 g/100 L were obtained by Pontes *et al.* (2006).

4.6.3 Total polyphenol content (TPC)

The total phenolic content of the ethanolic extracts were calculated by using calibration curve shown in Appendix C obtained from the Gallic acid solutions ranging from 40 μg/ml to 70 μg/ml. Based on the equation obtained, total phenolic content was calculated and expressed in mg GAE/g of dried extract. The phenolic content is shown in Table 4.3. The total phenolic content of betel leaf extract blended rum of sample M', N', O', P' and Q' were

67.28, 65.12, 79.63, 85.19 and 89.51 mg GAE/L. The total phenolic content of betel leaf extract blended rum was found 89.51 mg GAE/L for sample Q' was maximum and 65.12 mg GAE/L for sample N' was minimum. Generally, the TPC increases with respect to increase in concentration of betel leaf extract. Similar concentration of total polyphenol content was obtained (Tagrida and Benjakul, 2020).

4.6.4 Antioxidant activity

The average DPPH activity (% inhibition) of betel leaf extract incorporated rum (M', N', O', P', Q') were found to be 58.5, 65.09, 67.92, 66.67 and 71.13, respectively. Tagrida and Benjakul (2020) showed the relation between different modes of antioxidant with extraction of betel leaf.

4.6.5 Optimization study

As regard with the sensory scores and chemicals analysis, the optimization study was carried out as given in Table 4.7.

The optimized goals for treatment are shown in the Table 4.7

Table 4.7 Optimized goals for treatment (sensory)

Parameters	Optimized treatment
Color	O'
Flavor	O'=P'
Taste	O'
After taste	O'
Overall acceptance	O'

Regarding the sensory parameters, treatment O', i.e., 0.9% was found to be superior betel leaf extract blended rum. The optimized goals for chemical treatment are shown in Table 4.8.

Table 4.8 Optimized goals for treatment (chemical analysis)

Parameters	Optimized treatment	Remarks
Methanol (g/100 L)	O' (36.83)	In the range
Fusil oil (g/100 L)	O' (144.5)	In the range
Total polyphenol (mg GAE/L)	O' (79.63)	In the rage of health benefit
Antioxidant (% inhabitation)	O' (67.92%)	In the rage of health benefit

From the study of different parameters, treatment O' treated with 0.9% betel leaf extract incorporate rum had superior sensory characteristics. Similarly, Methanol and fusil oil is in the range and antioxidant and total polyphenol are in the health benefit range.

Part V

Conclusions and recommendations

5.1 Conclusions

Based on the results and discussion, the following points can be concluded.

1. Herbal rum of acceptable sensory quality of desirable chemical/ characteristics can be prepared by incorporating *gurjo* (*Tinospora cordifolia*) and betel (*Piper betle*) leaf extracts.
2. The optimum amounts of *gurjo* and betel leaf extracts for herbal rum of 40% ABV are 0.15% and 0.9% respectively.
3. In the optimization treatment of *gurjo* extract incorporated rum, the value of methanol, fusel oil, TPC and antioxidant activity were found 33.52 mg/L, 143.5 mg/L, 12.07 mg GAE/L and 73.27%, respectively.
4. In the optimization treatment of betel leaf extract incorporated rum, the value of methanol, fusel oil, TPC and antioxidant activity were found 36.83 mg/L, 144.5 mg/L, 79.69 mg GAE/L and 67.92%, respectively.

5.2 Recommendations

1. Amount greater than 0.15% *gurjo* extract incorporated rum cannot be used because of undesirable taste.
2. Amount greater than 0.9% betel leaf extract incorporated rum cannot be used because of undesirable taste.
3. Potential utilization of other herbs on quality of rum can be studied.
4. Extraction and blending of herbs during fermentation can be studied.
5. Phytochemical properties of the aged herbal rum can be studied.

Part VI

Summary

Herbal rum or spiced rum is now produced in different countries by fermentation of molasses and adding either oleoresin, herbs or spices. It is one of the best ways of utilizing these valuable compounds, such as antioxidants, found in herbs. Herbs can also be used to increase ester content, which is essential for desirable flavor of the product.

For rum preparation, molasses collected from local market was fermented at ambient condition for 9 days with *Saccharomyces cerevisiae* and the broth (wash) pot-distilled to obtain alcohol. The distillate was maintained to 40% ABV and alcoholic extracts of herbs (*gurjo* and betel leaves) were added at various concentrations. The formulated samples were then optimized through sensory evaluation and analyzed for chemical parameters (methanol, total polyphenol content, fusel oil, and antioxidant activity).

Rum containing 0.15% *gurjo* extract was found to be significantly superior ($p<0.05$) in terms sensory quality. The fusel oil, methanol, total polyphenol and antioxidant activity were 143.5 mg/L, 33.52 mg/L, 12.07 mg GAE/L and 73.27% inhibition (DPPH assay), respectively. Similarly, rum containing 0.9% betel leaf extract was significantly superior in terms of sensory quality. The fusel oil, methanol, total polyphenol and antioxidant activity were 144.5 mg/L, 36.83 mg/L, 79.69 mg GAE/L and 67.92 % inhibition, respectively. The selected herbal rums had desirable sensory characteristics and the chemical parameters were within legal standard for rum. The study shows that it is possible to prepare herbal rums with beneficial properties (e.g., antioxidant- and improved flavor properties) by utilizing extracts from locally available herbs like *gurjo* and betel leaves.

References

Agrawal, A., Malini, S., Bairy, K. L. and Rao, M. S. (2002). Effect of *Tinospora cordifolia* on learning and memory in normal and memory deficit rats. *Indian J. Pharmacol*. **34** (5), 339-349.

Alcarde, A. R., Souza, P. A. d. and Belluco, A. E. d. S. (2011). Chemical profile of sugarcane spirits produced by double distillation methodologies in rectifying still. *Ciência e Tecnologia de Alimentos*. **2** (31), 355–360. [doi:10.1590/s0101-20612011000200012]

Alexander, C. P. and Kirubakaran, C. J. (2010). Water soluble fraction of *Tinospora cordifolia* leaves enhanced the non-specific immune mechanisms and disease resistance in Oreochromis mossambicus. *Fish & Shellfish Immunol.* **29** (5), 765-772. [doi:10.1016/j.fsi.2010.07.003].

Ambrose, D. C. P., Manickavasagan., A. and Naik, R. (2016). "Leafy Medicinal Herbs: Botany, Chemistry, Postharvest Technology and Uses" (1st ed.). CABI.

Andrew, G. H. and John, R. P. (2003). "Handbook of Animal-Based Fermented Food and Beverage Technology" (2nd ed.).Kluwer Academic. London.

Andrew, G. H. and Piggott, N. (2003). "Fermented Beverage Production" (2nd ed.). Springer Science & Business Media. USA. [ISBN 0306472759].

Anjum, A., Vani, C. and Madhavan, N. R. (2020). Antioxidant activity of eugenol in *Piper betel* leaf extract. *J. Family Med. Prim. Care*. **9** (1), 327-331. [doi: 10.4103/jfmpc.jfmpc_809_19].

Anonymous. (2010). "Production Process of Rum". US Government. Retrieved from http://www.law.cornell.edu/cfr/text/27/5.22. [Accessed 02 september, 2022].

AOAC. (2005). "Official Methods of Analysis of AOAC International" (18th ed.). Suite 500, USA. AOAC International.

Arroyo, R. (2015). Rafael arroyo's lost papers on rum. Retrieved from https://www.bostonapothecary.com/rafael-arroyos-lost-papers-on-rum/. [Accessed 07 June 2022].

Asthana, J. G., Jain, A., Mishra, A. and Vijaykanth, M. S. (2001). Evaluation of antileprotic herbal drug combinations and their combination with Dapsone. *Indian Drugs-Bombay*. **38** (2), 82-86.

Atal, C. K., Dhar, K. L. and J., S. (1975). The chemistry of Indian *Piper* species. *Lloydia.* **38** (3), 256-264.

Azahar, N. I., Mokhtar, N. M. and Arifin, M. A. (2020). *Piper betle:* a review on its bioactive compounds, pharmacological properties, and extraction process. *IOP Conference Series: Materials Sci. and Engineering,* . **991**. [doi:10.1088/1757-899x/991/1/012044].

Bajpai, V., Pandey, R., Negi, M. P. S., Bindu, K. H., Kumar, N. and Kumar, B. (2012). Characteristic differences in metabolite profile in male and female plants of dioecious *Piper betel* L. *J Biosci.* **37** (1), 1061-1066.

Bajpai, V., Sharma, D., Kumar, B. and Madhusudanan, K. P. (2010). Profiling of *Piper betel* L. cultivars by direct analysis in real time mass spectrometric technique. *Biomed Chromatogr.* **24** (12), 1283-1286.

Balami, N. P. (2004). Ethnomedicinal uses of plants among the Newar community of pharping village of Kathmandu District, Nepal. *TU J.* **24** (1), 13-19. [doi:10.3126/tuj.v24i1.251].

Basireddy, S., Bernard, A. R., D, S., Meda, S., K Himakar, R. and G, S. (2019). Antifungal activity of solvent extracts of *Piper betel* and *Ocimum sanctum* Linn on *Candida albicans*: An in vitro comparative study. *J. Oral. Maxillofac. Pathol.* **23** (3), 333-337. [doi: 10.4103/jomfp.JOMFP_167_19].

Berketova, L. V., Kryukova, E. V., Goryacheva, E. D., Ilyuchina, N. V. and Kolokolova, A. Y. (2020). Evaluation of using herbal extracts in enriched beverages' production *Earth and Environ. Sci.* **640** (2021). [doi:10.1088/1755-1315/640/2/022068].

Bhat, K. S., Thomas, J., Kempraj, V., Danesh, A. and Sridhara, B. Y. (2015). Betel vine leaf extract inhibits mildew fungus of nyctanthes arbor-tristis growth under in vitro conditions. *Int. J. Plant Pathol.* **6**, 1-7. [doi:10.3923/ijpp.2015].

Bhatt, R. P. and Sabnis, S. D. (1987). Contribution to the ethnobotany of Khedbrahma region of North Gujarat. *J. Econ. Taxon. Bot.* **9**, 138-145.

Biney, R. P., Owusu, A. P. E., Ameyaw, E. O., Afortude, J. K., Henneh, I. T., Obese, E., Nyarko, S. B. and Ekor, M. (2020). Effects of three herb-based alcoholic beverages manufactured in Ghana on sperm characteristics and reproductive hormones in rats. *Scientific Afr.* **7**, e00316.

Bishayi, B., Roychowdhury, S., Ghosh, S. and Sengupta, M. (2002). Hepatoprotective and immunomodulatory properties of *Tinospora cordifolia* in CCl_4 intoxicated mature albino rats. *J. Toxicol. Sci.* **27** (3), 139-146.

Biswasroy, P., Panda, S., Das, C., Das, D., Kar, D. M. and Ghosh, G. (2020). *Tinospora cordifolia*– A plant with Spectacular natural immunobooster. *Res. J. Pharm. Tech.* **12** (2), 1035-1038. [doi: 10.5958/0974-360X.2020.00190.0].

Blois, M. S. (1958). Antioxidant determinations by the use of stable free radical. *Nature*. **81**, 1199-2000.

Bluhm, L. (1983). Distilled beverages, Biotechnology. **5**, 447-475. [Cited in J. J. (Rehm, & Reed, G, Eds). Vele Chimie Weinheim, London].

Broom, D. (2003). "Rum" (1st ed.). Abbeville Press Inc. U.S.

Carioca, J. O. B. (2011). Ethanol production from sugar-based feedstocks. *Comprehensive Biotechnology* 27–35. [doi:10.1016/B978-0-08-088504-9.00184-7].

Chan, E. and Wong, S. (2014). Phytochemistry and pharmacology of three piper species: an update. *Int. J. Pharmacogn*. **1** (9), 534-544.

Chandrasekaran, C. V., Mathuram, L. N., Daivasigamani, P. and Bhatnagar, U. (2009). *Tinospora cordifolia*, a safety evaluation. *Toxicol. Invitro.* **23** (7), 1220-1226. [doi:10.1016/j.tiv.2009.07.030].

Chavan, T., Ghadge, A., Karandikar, M., Pandit, V. and Ranjekar, P. (2017). Hepatoprotective activity of Satwa, an ayurvedic formulation against alcohol-induced liver injury in rats. *Altern. Ther. Health Med.* . **23** (4), 34-40.

Chaveerach, A., Mokkamul, P., Sudmoon, R. and Tanee, T. (2006). Ethnobotany of the genus *Piper* (Piperaceae) in Thailand. *Ethnobotany Res Appl.* **4**, 223-231.

Chibber, H. M. (1913). The morphology and histology of *Piper betel*, Linn.(the Betel-vine). *Bot J Linn Soc.* **41** (283), 357-383.

Clarke, M. A. (2003). Syrups *Encyclopedia of Food Sciences and Nutrition*. 5711-5717. [doi:10.1016/b0-12-227055-x/01175-5]

Communities, T. C. o. E. (1989). "Appellations of Origin (Spirit Drinks) Council Regulations". Retrieved from https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:31989R1576. [Accessed 18 May, 2022].

Desai, V. R., Ramkrishnan, R., Chintalwar, G. J. and Sainis, K. B. (2007). G1-4A, an immunomodulatory polysaccharide from *Tinospora cordifolia*, modulates macrophage responses and protects mice against lipopolysaccharide induced endotoxic shock. *Int. Immunopharmacol*. **7** (10), 1375-1386. [doi:10.1016/j.intimp.2007.06.004].

Destruhaut, C., Fahrasmane, L. and Parfait, A. (1986). "Technologie rhumiere. Etude des rhums de sirop". Institute of Food Science and Technology. London.

Durani, L. W., Khor, S. C., Tan, J. K., Chua, K. H., Mohd Yusof, Y. A. and Makpol, S. (2017). *Piper betel* L. modulates senescence-associated genes expression in

replicative senescent human diploid fibroblasts. *Biomed. Res. Int.* . 1-9. [doi:10.1155/2017/6894026].

Dwivedi, V. and Tripathi, S. (2014). Review study on potential activity of *Piper betle*. *J. Pharmacogn Phytochem.* **3** (4), 93-98.

EAC. (2001). "East African Standard Rum-Specification" (**1st** ed.). Retrieved from https://law.resource.org/pub/eac/ibr/eas.146.2000.html. [Accessed 08 Sept, 2022].

Fahrasmane, L. and Ganou-Parfait, B. (1998). Microbial flora of rum fermentation media. *J. Appli. Microbiol.* . **84**, 921-928.

Fahrasmane, L. and Parfait, A. (2003). A. Rum. 5021-5027. (2nd ed.). *In Ency. Food Sci. Nutri.* Academic Press: Oxford, UK].

FSANZ. (2000). "Standard 2.7.5—spirits". FSANZ: Canberra, Australia,. FSANZ (Food Standards Australia & New Zealand).

Ganie, S. A., Upadhyay, N. C., Agnihotri, R. K. and Sharma, R. K. (2013). Studies on antioxidant activity and total phenolic content of *Tinospora cordifolia* (Miers.) stem using invitro models. *Am. J. Phytomed. Clin. Therapeut.* **1**, 617-627.

Garg, S. C. and Jain, R. (1996). Volatile constituents of the essential oil of *Piper betel L.* (Cultiver Sagar Bangla). *Indian. J. Chem., Sect .B Organ. Chem.* **35** (8), 874-875.

Green, V. (2015). The microbial ecology of a rum production process. Ph.D Thesis Univ. New South Wales School of Chemical Science and Engineering, Australia.

Ickes, C. M. and Cadwallader, K. R. (2018). Effect of ethanol on flavor perception of rum. *Food Sci. Nutr.* **6** (4), 912-924. [doi:10.1002/fsn3.629].

Ilaiyaraja, N. and Khanum, F. (2011). Antioxidant potential of *Tinospora cordifolia* extracts and their protective effect on oxidation of biomolecules. *Pharma. J.* **3**, 56-62. [doi:10.5530/pj.2011.20.11].

Jana, U., Chattopadhyay, R. N. and Shw, B. P. (1999). Preliminary studies on anti-inflammaory activety of *Zingiber officinale* Rosc., *Vitex negundo* Linn. and *Tinospora cordifolia* (Wilid) Miers in albino rats. *Indian J. Pharmacol.* . **31** (3), 232.

Jeffery, J. D. E. (2012). Aging of whiskey spirits in barrels of non-traditional volume. M. Tech. USA. Michigan State Univ.,

Kalikar, M. V., Thawani, V. R., Varadpande, U. K., Sontakke, S. D., Singh, R. P. and R.K., K. (2008). Immunomodulatory effect of *Tinospora cordifolia* extract in

human immunodeficiency virus positive patients. *Indian J. Pharmacol*. **40** (3), 107-110. [doi:10.4103/0253-7613.42302].

Kampen, W. H. (1975). Technology of the rum industry sugar. *J. Food Sci.* **2**, 36-43.

Kanjwani, D. G., Marathe, T. P., Chiplunkar, S. V. and Sathaye, S. S. (2008). Evaluation of immunomodulatory activity of methanolic extract of *Piper betel*. *Scandinavian J. Immuno.* **67** (6), 589-593. [doi:10.1111/j.1365-3083.2008.02110.x].

Kapur, P., Jarry, H., Wuttke, W., Pereira, B. M. J. and Wuttke, D. S. (2008). Evaluation of the antiosteoporotic potential of *Tinospora cordifolia* in female rats. *Maturitas*. **59** (4), 329-338.

Karkal, Y. R. and Bairy, L. K. (2007). Safety of aqueous extract of *Tinospora cordifolia* (TC) in healthy volunteers: a double blind randomised placebo controlled study. *Iranian J. Pharmacol. Ther.* **6** (1), 59-61.

KC, J. B. and Rai, B. K. (2007). "Basic Food Analysis Handbook" (1st ed.). Anamnagar, Kathmandu-32, Nepal. [ISBN 978-99949-2-796-7].

Khare, C. P. (2007). "Indian Medicinal Plants- An Illustrated Dictionary". Vol. 663. Springer Science and Business Media, LLC. New York, U.S.A.

Langrand, J., Regnault, H., Cachet, X., Bouzidi, C., Villa, A. F., Serfaty, L., Garnier, R. and Michel, S. (2014). Toxic hepatitis induced by a herbal medicine: *Tinospora crispa*. *Phytomed.* **21** (8-9), 1120-1123. [doi:10.1016/j.phymed.2014.04.031].

Lehtonen, M. and Suomalainen, H. (1977). "The Microbiology of Alcoholic Beverages". Academic Press. London.

Lino, F. S. d. O., Basso, T. O. and Sommer, M. O. A. (2018). A synthetic medium to simulate sugarcane molasses. *Biotechno. Biofuels.* [doi:10.1186/s13068-018-1221-x].

Madhumita, M., Guha, P. and Nag, A. (2019). Extraction of betel leaves (*Piper betle* L.) essential oil and its bio-actives identification: Process optimization, GC-MS analysis and anti-microbial activity. *Ind. Crops Prod.* ***138***, 1-12. [doi: 10.1016/j.indcrop.2019.111578].

Madrera, R. R., Gomis, D. B. and Alonso, J. J. M. (2003). Influence of distillation system, oak wood type, and aging time on volatile compounds of cider brandy. *J. Agric. Food Chem.* **51** (19), 5709–5714. [doi:10.1021/jf034280o]

Mahima, Rahal, A., Prakash, A., Verma, A. K., Kumar, V. and Roy, D. (2014). Proximate and elemental analyses of *Tinospora cordifolia* stem. *Pak. J. Biol. Sci*. **17** (5), 744–747. [doi: 10.3923/pjbs.2014.744.747].

Mangwanda, T., Johnson, J. B., Mani, J. S., Jackson, S., Chandra, S., McKeown, T., White, S. C. and Naiker, M. (2021). Processes, challenges and optimisation of rum production from molasses—a contemporary review. *Fermentation*. **7** (1). [doi:10.3390/fermentation7010021].

Medeiros, A. B. P., deMatos, M. E., dePinho Monteiro, A., de Carvalho, J. C. and Soccol, C. R. (2017). Cachaça and rum. *In:* "Current Developments in Biotechnology and Bioengineering".). pp. 451-468. Elsevier.

Meshram, A., Bhagyawant, S. S., Gautam, S. and Shrivastava, N. (2013). Potential role of *Tinospora cordifolia* in Pharmaceuticals. *World J. Pharm. Pharm. Sci.* **2** (6), 4615-4625.

Mittal, J., Sharma, M. M. and Batra, A. (2014). *Tinospora cordifolia:* a multipurpose medicinal plant. *J. Med. Plants.* **2** (2), 32-47.

Modi, B., Koirala, N., Aryal, S. P., Shrestha, J., Koirala, S., Upadhyaya, J., Basnyat, R. C., Nassan, M., A.,, Alqarni, M. and Batiha, G. E. (2021). *Tinospora cordifolia* (Willd.) Miers:phytochemical composition, cytotoxicity, proximate analysis and their biological activities. *Cell. Mol. Biol.* . **67** (1), 1-8. [doi:10.14715/cmb/2021.67.1.8].

More, P. and Pai, K. (2012). In vitro NADH-oxidase, NADPH-oxidase and myeloperoxidase activity of macrophages after *Tinospora cordifolia* (guduchi) treatment. *Immunotoxicol*. **34** (3), 368-372. [doi:10.3109/08923973.2011].

Mosedale, J. R. (1995). Effects of oak wood on the maturation of alcoholic beverages with particular reference to whisky. *Forestry*. **68** (3), 203-230.

Murtagh, J. (1995). "Molasses as a feedstock for alcohol production". Nottingham University Press. Nottingham.

Murtagh, J. E. (1974). Feedstocks, fermentation and distillation for production of heavy and light rums. 243-256. [Cited in D. W. Clutton. "Rum The Flavour Industry". Virginia, USA.

Murtagh, J. E. (2003). Feedstocks, fermentation and distillation for production of heavy and light rums. 243–255. [Cited in D. Kelsall, K. Jacques and T. Lyons. "In The Alcohol Textbook " (4th ed.). Nottingham University Press. Nottingham, UK,].

Muruganandam, L., Krishna, A., Reddy, J. and Nirmala, G. S. (2017). Optimization studies on extraction of phytocomponents from betel leaves. *Resour Effic Technol.* **3** (4), 385-393.

Nalina, T. and Rahim, Z. H. A. (2007). The crude aqueous extract of *Piper betel* L. and its antibacterial effect towards *Streptococcus mutans*. *Am. J. Biotechnol. Biochem.* **3** (1), 10-15.

Nasreen, S., Radha, R., Jayshree, N., Selvaraj, B. and Rajendran, A. (2010). Assessment of quality of *Tinospora cordifolia* (Willd) Miers pharmacognostical and phyto-physicochemical profile. *Int. J. Compr. Pharm* **1** (5), 1-4.

Nayampalli, S. S., Ainapure, S. S., Samant, B. D., Kudtarkar, R. G., Desai, N. K. and Gupta, K. C. (1988). A comparative study of diuretic effects of *Tinospora cordifolia* and hydrochlorothiazide in rats and a preliminary phase I study in human volunteers. *J. Postgraduate Med.* **34** (4), 233.

Neeraja, P. V. and Margaret, E. (2013). Amruthavalli (*Tinospora cordifolia*) multipurpose rejuvenator. *Int. J. Pharm. Bio. Chem. Sci.* **3** (2), 233-241.

Nicol, D. A. (2003). Rum. [Cited in A. G. H. Lea and J. R. Piggott. "Fermented Beverage Production " (2nd ed.). Kluwer Academic New York].

Nile, S. H. and Khobragade, C. N. N. (2009). Determination of nutritive value and mineral elements of some important medicinal plants from western part of India. *J. Med. Plants*. **8**, 79-88.

NiMade, D. M. W. N., MariaMalida, V. S., DwiArymbhi, S., PutuEra, S. K. Y., NiLuh, K. A. A. D., Erna, C. and Rika, H. (2021). *Piper betel* (L): Recent review of antibacterial and antifungal properties, safety profiles, and commercial applications. *Molecules*. **26** (8), 2321. [doi: 10.3390/molecules26082321].

Nykaenen, L. and Suomalainen, H. (1983). "Aroma of Beer, Wine and Distilled Alcoholic Beverages". Berlin: Akademie-Verlag.

Oliveira, A. P., Ferreira, J. G., Riboira, S., Andrade, P. B. and Valentão, P. (2016). Bioactive natural products from *Piper betel* L. leaves and their α-glucosidase inhibitory potential. *Rec. Nat. Prod.* **10** (6), 771.

Onkar, P., Bangar, J. and Karodi, R. (2012). Evaluation of Antioxidant activity of traditional formulation Giloy satva and hydroalcoholic extract of the *Curculigo orchioides*. *J. Appl. Pharm. Sci*. **02** (06).

Paine, A. J. and Dayan, A. D. (2001). Defining a tolerable concentration of methanol in alcoholic drinks. *Human & Experimental Toxicology*. **20** (11), 563–568. [doi:10.1191/096032701718620864]

Panchabhai, T. S., Kulkarni, U. P. and Rege, N. N. (2008). Validation of therapeutic claims of *Tinospora cordifolia*: a review. *Phytother. Res.* **22** (4), 425-441.

Panda, S., Sharma, R. and Kar, A. (2018). Antithyroidic and hepatoprotective properties of high-resolution liquid chromatography–Mass spectroscopy-standardized *Piper betle* leaf extract in rats and analysis of its main bioactive constituents. *Pharmacognosy Mag.* **14**, 658-664.

Parthasarathy, U., Asish, G. R., Saji, K. V., Johnson, G. K., Leela, N. K. and Mathew, P. A. (2014). Diversity study of leaf volatile oil constituent of *Piper* species based on GC/MS and spatial distribution. *J. Spices Aromat. Crops.* **23** (1), 10-16.

Patel, M. B. and Mishra, S. (2011). Hypoglycemic activity of alkaloidal fraction of *Tinospora cordifolia*. *Phytomed.* . **18** (2), 1045-1052.

Patil, R. S., Harale, P. M., Shivangekar, K. V., Kumbhar, P. P. and Desai, R. R. (2015). Phytochemical potential and in vitro antimicrobial activity of *Piper betle* Linn. leaf extracts *J. Chem. Pharm.* **7** (5), 1095-1101.

Paturau, J. M. (1969). "By-Products of the Cane Sugar Industry: An Introduction to their Industrial Utilization". Elsevier Publishing Company. [ISBN 9780444407375].

Piggott, J. R. and Conner, J. M. (2003). Whiskies. *In. Ferm. Bevera. Prod.*, 239-262.

Piggott, J. R., Lea, G. H., Paterson, A., Swanston, J. S., Andrew, G. H. L. and Andrew, J. (2003). "Fermented Beverage Production " (2nd ed.). Kluwer Academic. New York.

Pin, K. Y., Chuah, T. G., Rashih, A. A., Law, C. L., Rasadah, M. A. and Choong, T. S. Y. (2009a). Drying of betel leaves (*Piper betel* L.): quality and drying kinetics. *Drying Technol.* **27** (1), 149–155. [doi:10.1080/07373930802566077]

Pin, K. Y., LuqmanChuah, A., AbdullRashih, A., Rasadah, M. A., Law, C. L. and Choong, T. S. Y. (2009b). Solid-liquid extrection of Betel leaves (*Piper betle* L.). *J. Food Process Eng.* **34**, 548-565. [doi:10.1111/j.1745-4530.2009.00395.x].

Pontes, M. J. C., Santos, S. R. B., Araújo, M. C. U., Almeida, L. F., Lima, R. A. C., Gaião, E. N. and Souto, U. T. C. P. (2006). Classification of distilled alcoholic beverages and verification of adulteration by near infrared spectrometry. *Food Res. Int.* **39** (2), 182-189. [doi:10.1016/j.foodres.2005.07.005].

Pounder, P. (2010). Branding: a caribbean perspective on rum manufacturing competitiveness. *Int. J. Entrep. Small Bus.* **9**, 394-406.

Pradhan, D., Suri, K. A., Pradhan, D. K. and Biswasroy, P. (2013). Golden heart of the nature: *Piper betel* L. *J. Pharmacog. Phytochem.* **1** (6), 147-167.

Prakash, B., Shukla, R., Singh, P., Kumar, A., Mishra, P. K. and Dubey, N. K. (2010). Efficacy of chemically characterized *Piper betel* L. essential oil against fungal and

aflatoxin contamination of some edible commodities and its antioxidant activity. *Int. J. Food Microbiol.* **142** (1-2), 114-119.

QuesadaGranados, J., MerelowGuervos, J. J., OliverasLopez, M. J., GonzalezPenalver, J., OlallaHerrera, M., BlancaHerrera, R. and LopezMartinez, M. C. (2002). Application of artificial aging techniques to samples of rum and comparison with traditionally aged rums by analysis with artificial neural nets. *J. Agri. Food Chem.* **50** (6), 1470-1477.

Raghu, R., Sharma, D., Ramakrishnan, R., Khanam, S., Chintalwar, G. J. and Sainis, K. B. (2009). Molecular events in the activation of B cells and macrophages by a non-microbial TLR4 agonist, G1-4A from *Tinospora cordifolia*. *Immunol. Lett.* . **123** (1), 60-71. [doi:10.1016/j.imlet.2009.02.005].

Rahal, A., Verma, A. K., Kumar, V. and Roy, D. (2014). Proximate and elemental analyses of *Tinospora cordifolia* stem. *Pak. J. Biol. Sci.* **17** (5), 744-747. [doi:10.3923/pjbs.2014.744.747].

Rai, B. K. (2012). "Essential of Industrial Microbiology".[ISBN 978-1-300-13701-6].

Rai, B. K. and Subba, D. K. (2016). "Basic Practical Manual on Industrial Microbiology" (1st ed.). Lulu Publication, USA.

Rai, K. K. R., Trivedi, R. V. and Umekar, M. J. (2019). Review on betel leaf used in various ailments. *Int. J. Pharmacognosy*. **6** (8), 259-267.

Rajgopal, P. L., Dhilna, K. K., Sajith Kumar, P. N. and John, J. (2013). Herbs in inflammation- a review. *Int. J. Ayu. Herb. Med.* **3** (4), 1289-1307.

Rani, J., Singh, L., Singh, H., Kapoor, M. and Singh, G. (2015). Priliminary phytochemical analysis of diferent solvent extracts from leaf and stem of *Tinospora cordifolia*. *Int. J. Phytoremediation*. **5**, 124-128.

Ravindran, P. N., Pillai, G. S., Balachandran, I. and Divakaran, M. (2012). Galangal. *In:* "Handbook of Herbs and Spices".). pp. 303-318. Elsevier.

Ricardo, V. (2011). Chemical profile of sugarcane spirits produced by double distillation methodologies in rectifying still. *J. Food Sci. Technol.* **32** (2), 335-360.

Sangeetha, M. K., Priya, C. D. and Vasanthi, H. R. (2013). Anti-diabetic property of *Tinospora cordifolia* and its active compound is mediated through the expression of Glut-4 in L6 myotubes. *Phytomed.* **20** (3-4), 246-248.

Sankhala, L., Saini, R. K. and Saini, B. S. (2012). A review on chemical and biological properties of *Tinospora cordifolia*. *Int. J. Med. Aromat. Plants*. **2**, 340-344.

Sarala, M., Velu, V., Anandharamakrishnan, C. and Singh, R. P. (2012). Spray drying of *Tinospora cordifolia* leaf and stem extract and evaluation of antioxidant activity. *J. Food Sci. Technol.* **49** (1), 119–122. [doi:10.1007/s13197-011-0364-6]

Sarma, C., Rasane, P., Kaur, S., Singh, J., Singh, J., Gat, Y. and Dhawan, K. (2018). Antioxidant and antimicrobial potential of selected varieties of *Piper betel* L. (betel leaf) *An. Acad. Bras. Ciênc.* **90**, 1-8. [doi:10.1590/0001-3765201820180285].

Satija, S., Bansal, P., Dureja, H. and Garg, M. (2015). Microwave assisted extraction of *Tinospora cordifolia* and optimization through central composite design. *J. Biol. Sci.* **15** (3), 106-115. [doi: 10.3923/jbs.2015.106.115].

Sawyer, H. E. (2005). The commercial analysis of cane molasses *J. Am. Chem. Soc.* **27** (6), 691-713. [doi:10.1021/ja01984a005]

Schoonees, B. M. and Pillay, V. (2004). A new filtration system for gum analysis in raw sugar and molasses *In. Proc. S. Afr. Sug. Technol. Ass.* . **78**.

Sharma, A. K., Gupta, A. and AmlaBatra, S. S. (2010). *Tinospora cordifolia* (Willd.) Hook. F. & Thomson - a plant with immense economic potential. *J. Chem. Pharm. Res*. **2** (5), 327-333.

Sharma, U., Bala, M., Kumar,, N., S., B.,, Munshi, R. K. and Bhalerao, S. (2012). Immunomodulatory active compounds from *Tinospora cordifolia*. *J. Ethnopharmacol*. **141**, 918-926. [doi:10.1016/j.foodres.2012.03.002].

Shrestha, T. and Lamichhane, J. (2021). Assessment of phytochemicals, antimicrobial, antioxidant and cytotoxicity activity of methanolic extract of *Tinospora cordifolia* (*Gurjo*). *Nepal J. Biotechnol.* . **9** (1), 18-23. [doi: 10.31216/njb.v9i1.38646].

Siddiqui, N., Rauf, A., Latif, A. and Mahmood, Z. (2017). Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-eZoofa (*Nepeta bracteata* Benth). *J. Taibah Univ. Med. Sci.* **12** (4), 360–363. [doi:10.1016/j.jtumed.2016.11.006].

Simmomds, C. (1994). Alcohol, its production, properties, chemistry, and industrial applications. **104** (2618), 431-432. [doi:431-432. 10.1038/104431a0].

Singh, S. S., Pandey, S. C., Srivastava, S., Gupta, V. S., Patro, B. and Ghosh, A. C. (2003). Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi). *Indian J. Pharmacol.* . **35** (2), 83-91.

Singla, A., Priya, M. A. and Singla, P. (2010). Review of biological activities of *Tinospora cordifolia*. *Webmed Cent. Pharm. Sci.* . **1** (9). [doi:10.9754/journal.wmc.2010.00606].

Sinha, K., Mishra, N. P., Singh, J. and Khanuja, S. P. S. (2004). *Tinospora cordifolia* (Guduchi), a reservoir plant for therapeutic application. *Indian J. Tradit. Knowl.* **3** (3).

Spandana, U., Ali, S. L., Nirmala,, T., S., M., and SipaiBabu, S. D. (2013). A review on *Tinospora cordifolia*. *Int. J. Curr. Pharma. Review Res.* **4** (2), 61-68.

Sundarraj, S., Thangam, R., Sreevani, V., Kaveri, K., Gunasekaran, P. and Achiraman, S. (2012). ϒ-Sitosterol from *Acacia nilotica* L. induces G2/M cell cycle arrest and apopyosis through c-Myc suppression inmMCF-7 and A549 cells. *J. Ethnopharmcol*. **141** (3), 803-809. [doi:10.1016/j.jep.2012.03.014].

Supavadee, B., Jirapon, T., Thanaporn, P., Teerapong, W., Piyarat, K., Benjaporn, B. and Tasana, P. (2019). Evaluation of antioxidant and anticancer effects of *Piper betle* L (Piperaceae) leaf extract on MCF-7 cells, and preparation of transdermal patches of the extract. *Trop. J. Pharm. Res.* **18** (6), 1265-1272.

Tagrida, M. and Benjakul, S. (2020). Ethanolic extract of Betel (*Piper betel* L.) and Chaphlu (*Piper sarmentosum* Roxb.) dechlorophyllized using sedimentation process: production, characteristics, and antioxidant activities. *J. Food Bioche.* **44**. [doi: 10.1111/jfbc.13508].

Thamaraikani, I. and Kulandhaivel, M. (2017). Purification of hydroxychavicol from *Piper betel* Linn and evaluation of antimicrobial activity against some food poison causing bacteria. *J. Pure Appl. Microbiol.* **11**, 1883-1889.

Thompson, L. (1992). Studies on the maturation of jamaican rum. Ph.D thesis. Univ. of the West Indies, Jamaica.

Toprani, R. and Patel, D. (2013). Betel leaf: revisiting the benefits of an ancient Indian herb. *South Asian J. Cancer*. **2** (3), 140–141. [doi: 10.4103/2278-330X.114120].

Tyagi, P., Chauhan, A. K. and Aparna. (2020). Optimization and characterization of functional cookies with addition of *Tinospora cordifolia* as a source of bioactive phenolic antioxidants. *LWT-Food Sci. Techn.* **130**. [doi:10.1016/j.lwt.2020.109639].

Upadhya, A. K., Kumar, K., Kumar, A. and Mishra, H. S. (2010). *Tinospora cordifolia* (Willd.) Hook. F. and Thoms. (Guduchi)—validation of the Ayurvedic pharmacology through experi mental and clinical studies. *Int. J. Ayurveda Res.* **1** (2), 112-121. [doi:10.4103/0974-7788.64405].

Upadhya, V., Hegde, H. V., Bhat, S., Hurkadale, P. J., Kholkute, S. D. and Hegde, G. R. (2012). Ethnomedicinal plants used to treat bone fracture from North-Central

Western Ghats of India. *J. Ethnopharmacol*. **142** (2), 557-562. [doi:10.1016/j.jep.2012.05.051].

Upadhyay, A., Kumar, K., Kumar, A. and Mishra, H. (2010). *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (*Guduchi*) - validation of the Ayurvedic pharmacology through experimental and clinical studies. *Int. J. Ayurveda Res.* **1** (2), 112. [doi:10.4103/0974-7788.64405]

Upadhyaya, R., Pandey, R. P., Sharma, V. and Verma Anita, K. (2011). Assessment of the multifaceted immunomodulatory potential of the aqueous extract of *Tinospora cordifolia*. *Res. J. Chem. Sci.* **1** (6), 71-76.

Vashist, N., Drabu, S., Nand, P. and Arora, P. (2011). Treatment strategies for monkey malaria. *Res. J. Pharm. Bio. Chem. Sci.* . **2** (4), 478-487.

Vázquez-Fresno, R., Rosana, A. R. R., Sajed, T., Onookome-Okome, T., Wishart, N. A. and Wishart, D. S. (2019). Herbs and spices- biomarkers of intake based on human intervention studies – a systematic review. *Genes & Nutrition*. **14** (1). [doi:10.1186/s12263-019-0636-8].

Vikash, C., Shalini, T., Verma, N. K., Singh, D. P., Chaudhary, S. K. and Asha, R. (2012). *Piper betel* phytochemistry, traditional use & pharmacological activity a review. *Int. J. Pharm. Res. Dev.* **4** (04), 216-223.

Wankat, P. (2007). "Separation Process Engineering: Including Mass Transfer Analysis" (3rd ed.). Prentice Hall. USA.

WendyVoon, W. Y., Ghali, N. A., Rukayadi, Y. and MeorHussin, A. S. (2014). Application of betel leaves (*Piper betle* L.) extract for preservation of homemade chili bo. *Int. Food Res. J.* **21** (6), 2399-2403.

Xu, F., Yu-kui, R. and Wei, T. (2010). Monitoring of nine trace elements in Chinese herbal medicine *Tinospora sinensis* (Lour.) Merr by ICP-MS. *Asian J. Chem*. **22** (9), 7429-7431.

Yildirim, H. K. (2021). Insights into the role of yeasts in alcoholic beverages. *Microbial Biotechnol. Food Health*. 21–52. [doi:10.1016/B978-0-12-819813-1.00002-5]

Yokota, M. and Fagerson, I. S. (1971). The major volatile components of cane molasses. *J. Food Sci.* **36** (7), 1091-1093. [doi:10.1111/j.1365-2621.1971.tb03353.x]

Zhang, H. S., Zhang, L. M. and Zhang, H. H. (2010). The observation of curative effect of knee ankylosis after injury auxiliary through huoxue Shujin Xunxi recipe. *Shaanxi J. Traditional Chinese Med.* **31** (8), 997-998. [doi:10.7666/d.y351065].

Zhao, T. F., Wang, X., Rimando, A. M. and Che, C. (1991). Folkloric medicinal plants: *Tinospora sagittata* var. *cravaniana* and *Mahonia bealei*. *Planta Medica.* **57** (5), 505. [doi:10.1055/s-2006-960188].

Appendices

Appendix A

Specimen Card for sensory evaluation

Hedonic Rating test

Date: -……

Name of panelist: -……

Please evaluate the Rum samples and indicate how much you like or dislike it for appearance, aroma, taste, after taste and overall acceptability on ranking 1-9 ranking scale.

1= Dislike extremely 2= Dislike very much 3= Dislike moderately

4= Dislike slightly 5= Neither like nor dislike 6= Like slightly

7= Like moderately 8= Like very much 9= Like extremely

Sample	Appearance	Aroma	Taste	Aftertaste	Overall acceptance
A					
B					
C					
D					
E					
F					

Comment If Any: …….

……..

Signature: _____

Appendix B

ANOVA for sensory analysis of product 1

Table B.1.1 Two-way ANOVA (no blocking) for appearance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	8.11222	2.7041	12.43	<.001
Panelist	8	0.3750	0.0469	0.22	0.985
Residual	24	5.2228	0.2176		
Total	35	13.7100			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.2 Two-way ANOVA (no blocking) for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	6.7031	2.2344	6.40	0.002
Panelist	8	3.3200	0.4150	1.19	0.347
Residual	24	8.3844	0.3494		
Total	35	18.4075			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.3 Two-way ANOVA (no blocking) for taste.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	5.6519	1.8840	7.52	0.001
Panelist	8	1.7050	0.2131	0.85	0.569
Residual	24	6.0106	0.2504		
Total	35	13.3675			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.4 Two-way ANOVA (no blocking) aftertaste.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	8.4542	2.8181	12.67	<.001
Panelist	8	1.5839	0.1980	0.89	0.539
Residual	24	5.3383	0.2224		
Total	35	15.3764			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.5 Two-way ANOVA (no blocking) for overall acceptability.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	3.9497	1.3166	5.99	0.003
Panelist	8	1.2400	0.1550	0.70	0.684
Residual	24	5.2778	0.2199		
Total	35	10.4675			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

ANOVA for sensory analysis of product 2

Table B.1.1 Two-way ANOVA (no blocking) for appearance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	19.4352	4.8588	13.56	<.001
Panelist	9	2.2802	0.2534	0.71	0.699
Residual	36	12.9008	0.3584		
Total	49	34.6162			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.2 Two-way ANOVA (no blocking) for aroma

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	10.7592	2.6898	6.84	<.001
Panelist	9	8.4122	0.9347	2.38	0.032
Residual	36	14.1568	0.3932		
Total	49	33.3282			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.3 Two-way ANOVA (no blocking) for taste.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	16.6012	4.1503	5.77	0.001
Panelist	9	7.2512	0.8057	1.12	0.374
Residual	36	25.9068	0.7196		
Total	49	49.7595			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.4 Two-way ANOVA (no blocking) aftertaste.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	23.2252	5.8063	9.76	<.001
Panelist	9	6.5042	0.7227	1.22	0.316
Residual	36	21.4108	0.5947		
Total	49	51.1402			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.5 Two-way ANOVA (no blocking) for overall acceptability.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	26.5792	6.6448	7.67	<.001
Panelist	9	2.2882	0.2542	0.29	0.972
Residual	36	31.1728	0.8659		
Total	49	60.0402			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

ANOVA for sensory analysis of product 3

Table B.1.1 Two-way ANOVA (no blocking) for appearance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	9.3586	3.1195	12.81	<.001
Panelist	8	2.9450	0.3681	1.51	0.205
Residual	24	5.8439	0.2435		
Total	35	18.1475			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.2 Two-way ANOVA (no blocking) for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	8.6144	2.8715	17.01	<.001
Panelist	8	0.9072	0.1134	0.67	0.711
Residual	24	4.0506	0.1688		
Total	35	13.5722			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.3 Two-way ANOVA (no blocking) for taste.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	5.0564	1.6855	13.80	<.001
Panelist	8	2.1556	0.2694	2.21	0.064
Residual	24	2.9311	0.1221		
Total	35	10.1431			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.4 Two-way ANOVA (no blocking) aftertaste.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	10.6022	3.5341	22.04	<.001
Panelist	8	1.2256	0.1532	0.96	0.492
Residual	24	3.8478	0.1603		
Total	35	15.6756			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.5 Two-way ANOVA (no blocking) for overall acceptability.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	14.6364	4.8788	24.99	<.001
Panelist	8	1.7406	0.2176	1.11	0.389
Residual	24	4.6861	0.1953		
Total	35	21.0631			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

ANOVA for sensory analysis of product 4

Table B.1.1 Two-way ANOVA (no blocking) for appearance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	9.4800	2.3700	8.79	<.001
Panelist	9	0.8010	0.0890	0.33	0.959
Residual	36	9.7040	0.2696		
Total	49	19.9850			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.2 Two-way ANOVA (no blocking) for aroma

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	15.0800	3.7700	9.10	<.001
Panelist	9	1.18	0.1311	0.32	0.964
Residual	36	14.9200	0.4144		
Total	49	31.1800			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.3 Two-way ANOVA (no blocking) for taste.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	51.2248	12.8062	33.24	<.001
Panelist	9	3.5978	0.3998	1.04	0.430
Residual	36	13.8712	0.3853		
Total	49	68.6938			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.4 Two-way ANOVA (no blocking) aftertaste.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	61.3548	15.3387	35.45	<.001
Panelist	9	3.7168	0.4130	0.95	0.492
Residual	36	15.5772	0.4327		
Total	49	80.6488			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.5 Two-way ANOVA (no blocking) for overall acceptability.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	20.4592	5.1148	16.02	<.001
Panelist	9	4.1432	0.4604	1.44	0.207
Residual	36	11.4928	0.3192		
Total	49	36.0952			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Appendix C

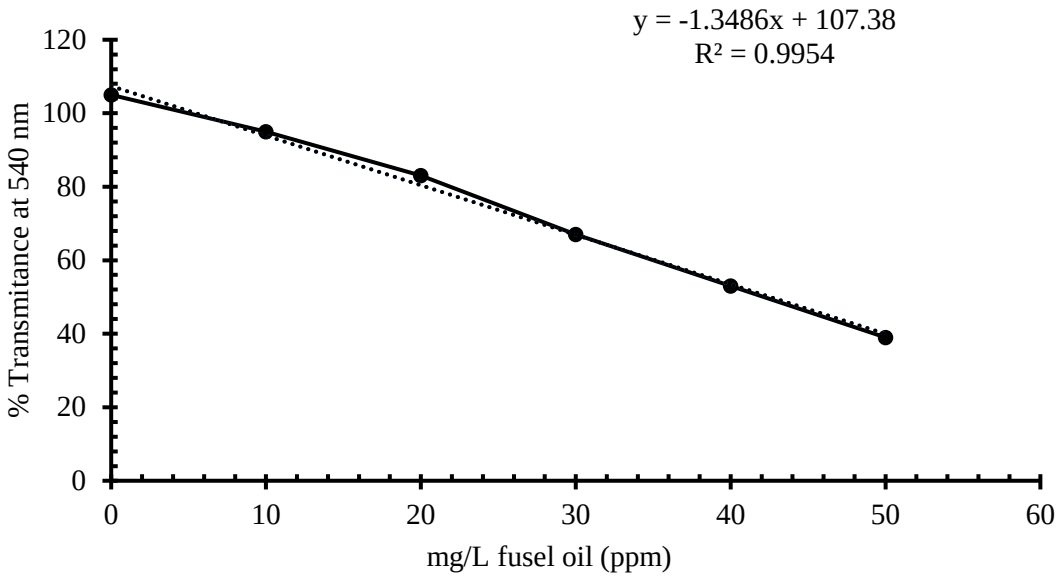

Fig. C 1 Standard curve for fusel oil content determination

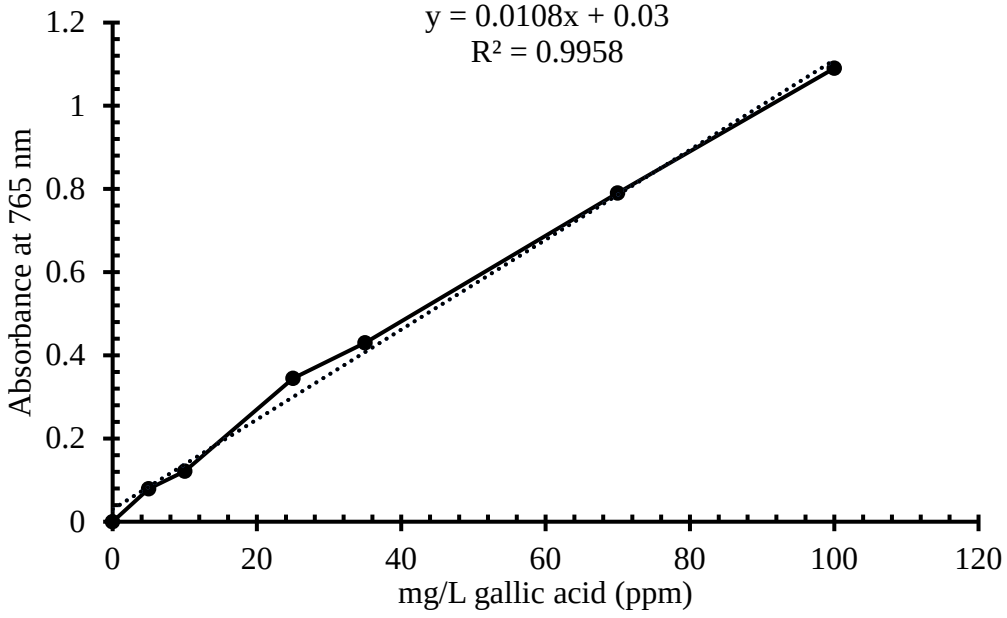

Fig. D 1 Standard curve for total phenolic content determination

Appendix D

One-way ANOVA

Table D.1.1 one-way ANOVA for fusel oil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	140.667	28.133	17.46	<.001
Residual	12	19.333	1.611		
Total	17	160.000			

Table D.1.1 One-way ANOVA for methanol

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	82.9363	16.5873	22.09	<.001
Residual	12	9.0102	0.7509		
Total	17	91.9465			

Table D.1.1 One-way ANOVA for polyphenol content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	14.3525	3.5881	16.30	<.001
Residual	10	2.2010	0.2201		
Total	14	16.5535			

Table D.1.1 One-way ANOVA for antioxidant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	25.1193	6.2798	10.67	0.001
Residual	10	5.8858	0.5886		
Total	14	31.0051			

One-way ANOVA

Table D.1.1 One-way ANOVA for fusel oil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	208.6333	41.7267	56.60	<.001
Residual	12	8.8467	0.7372		
Total	17	217.4800			

Table D.1.1 One-way ANOVA for methanol

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Source	5	53.5028	10.7006	40.13	<.001
Residual	12	3.2000	0.2667		
Total	17	56.7028			

Table D.1.1 One-way ANOVA for polyphenol content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	1395.519	348.880	96.89	<.001
Residual	10	36.008	3.601		
Total	14	1431.527			

Table D.1.1 One-way ANOVA for antioxidant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	262.530	65.632	41.09	<.001
Residual	10	15.973	1.597		
Total	14	278.502			

Color plates


P.1 Fermentation of rum


P.2 Distillation of ferment


P.3 Cleaning, cutting, drying and pulverizing of *gurjo*

P.4 Cleaning and drying of betel leaves

P.5 Preparation of determination of higher alcohols and methanol

P.6 Sensory evaluation of rum