Pharmaspire

RESEARCH ARTICLE

Formulation, evaluation, and antimicrobial study of immune-boosting herbal tea

Satyam Anand Singh, Yash Patel, Shreya Patel, Mane Ajay, Vishva Chauhan

Department of Pharmacognosy, ROFEL Shri G.M. Bilakhia College of Pharmacy, Vapi, Gujarat, India

ABSTRACT

Introduction: Herbal tea is caffeine free which boosts the immunity level and relieves stress. aim of study: To formulate and evaluate the immune-boosting herbal tea. Here, we made herbal tea from *Moringa oleifera* leaves and masked the bitter taste of *Moringa* with a natural sweetener and jaggery. **Material and Methods:** It is reported that jaggery boosts the immunity of the human body. *M. oleifera* is a versatile plant that has been traditionally used for its medicinal properties. *M. oleifera* has been shown to have anti- inflammatory, antimicrobial, antioxidant, and anticancer properties. Active chemical constituents of *M. oleifera* have been extracted by decoction method from the leaves. Other ingredients such as ginger, turmeric, black pepper, cardamom, and fennel were also mixed with *M. oleifera* and jaggery to formulate the tea. Cardamom and fennel were added to the tea for flavoring purpose and they also have the property of expectorant and carminative agent. **Result and discussion:** Due to the extremely low likelihood of herbal-herbal interactions, none of the substances interact with one another. It has been evaluated for various evaluation parameters, i.e., organoleptic test, physical parameters, phytochemical, chemical test, and antimicrobial test. The physical parameters of the tea were within the standard range which indicates that the granules of the tea are of optimum quality with optimum flowability. The presence of the secondary metabolite in the tea was also reported. The tea has stable pH and it was reported with good antimicrobial property against *Bacillus subtilis* and *Staphylococcus aureus*. Herbal tea was found acceptable to drink

KEY WORDS: *Bacillus subtilis*, Decoction, Kanamycin, *Moringa oleifera*, *Staphylococcus aureus*

INTRODUCTION

Herbal teas are regarded as a popular beverage globally. Many different herbs are combined to make herbal tea, also referred to as "tisanes." The dried leaves, seeds, nuts, barks, fruits, flowers, or other botanical components of herbal teas are combined to create tisanes.[1]

The *Moringa* plant is regarded as versatile because of its ability to provide edible food, oil, and purification of water for local communities. Despite their nutritious edible parts, *Moringa* products are sometimes classified as "famine food," consumed by humans in times of food scarcity. Asians who came to settle in the country in the late 18th Century introduced *Moringa* in Malawi. An essential aspect that may affect the growth and productivity of the *Moringa* species is the state of the soil. The species needs sandy or loamy soil that ranges in pH from 5 to 9, which is mildly acidic to alkaline. However, it will not thrive on soils that include a lot of clay. The use of cooked leaves with or without flowers as vegetables was recorded long time ago. The leaves contain antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids. They also contain high levels of iron such that doctors have reportedly prescribed them for anemic patients. Almost

Address for Correspondence:
Satyam Anand Singh,
E-mail: satyamanandsingh19@gmail.com

Access this article online

Website: http://isfcppharmaspire.com

DOI: 10.56933/Pharmaspire.2023.15121

Date of Submission: 23 August 2023
Date of Revision: 19 September 2023
Date of Acceptance: 13 October 2023

Table 1: Comparison between herbal tea, green tea, and black tea[9]

Herbal tea	Green tea	Black tea
Herbal tea is achieving more calm and relaxed state of mind.	Allergic reaction is shown due to consumption.	Black tea is a source of caffeine.
Herbal tea is caffeine free.	Consumption of green tea for longer period has also proven to relate to esophageal cancer.	Caffeine causes insomnia in adults, children, and infants.
They trigger immune system.	They trigger central nervous system.	They trigger central nervous system.
Herbal tea boosts energy levels and relieves stress.	Green tea is known to cause other less serious side effects such as heartburn, stomach upset, loss of appetite, diarrhea, etc.	Black tea is known to cause erosion of dental enamel if taken continuously.
It helps in stimulating the internal organs and promotes good sleep.	They help in weight loss by increasing the metabolism.	They may have a carcinogenic effect.
They do not go under oxidation process during production	They also do not undergo oxidation process during production.	They undergo oxidation process during production.

Table 2: In-house herbal tea prepared in two different combinations

Ingredients	F1 (40 g)	F2 (40 g)	For (100 g)
Moringa oleifera	18 g	18 g	45 g
Ginger	6 g	6 g	15 g
Black pepper	4 g	4 g	10 g
Cardamom	2 g	2 g	5 g
Jaggery	2 g	2 g	5 g
Resin	2 g	-	-
Fennel	-	2 g	5 g
Turmeric	6 g	6 g	15 g

all parts of the plant have recorded uses in medicinal preparations. Locally, crushed leaves are used for treating conjunctivitis, scorpion bites, and diarrhea. The roots are used for healing fevers in children, excising evil spirits and as a charm against witches.[2]

Different species of *Moringa* plant is *Moringa oleifera, Moringa ovalifolia, Moringa peregrina, Moringa. pygmaea, Moringa Rivae, Moringa Ruspoliana, Moringa Stenopetala, Moringa arborea, Moringa borziana, Moringa concanensis, Moringa drouhardii, Moringa hildebrandtii,* and *Moringa longituba.*[3] From all species, *M. oleifera* has high content of flavonoid and flavanol which are responsible for coloring and flavoring characteristics in herbal tea and it is widely cultivated in India and economical and for that reason, we selected *M. oleifera* for our research purpose.[4]

They are made up of single- or multiple-herbal ingredients that are brewed into a decoction or infusion and consumed for medicinal purposes.[5] The use and popularity of herbal teas appear to have increased gradually.[6] This might be connected to the well-known advantages of treating a variety of chronic conditions. As a result, herbal teas are a part of the fast-growing industry for wellness drinks.[7] The presence of diverse secondary metabolites, which are responsible for their pharmacological activity and health benefits, is a common characteristic of the herbal ingredients that go into the recipe. In fact, a lot of traditional recipes, particularly those for controlling chronic conditions, is now offered as herbal teas.[8]

In certain communities in Nigeria, the hot infusion of recipes containing *M. oleifera*, *Zingiber officinale,* and *Citrus* spp. is used for treating cold and cough. *M. oleifera*, which is often referred to as the miracle tree, is believed to treat over 300 diseases. In many communities in the Northern part of the country, it is used as a vegetable in soups and salads. The leaves of *M. oleifera* are an outstanding source of nutritionally rich vegetable because of its proven high content of amino acids, fats and omega oils, potassium, calcium, phosphorous, as well as other trace minerals, antioxidants, and anti-inflammatory substances. At present, coarsely powdered *M. oleifera* leaves are packed in tea bags as herbal tea to be brewed as infusions and are commercially available in many superstores in Nigeria.

A thorough review of the literature revealed the need to investigate the morphological, physicochemical, phytochemical, and quantitative aspects of the commercially available tea powders because the use of tea as a beverage and health drink needs to be encouraged. Tea is the most excellent way by which we can take many more herbal extracts in a sip of tea since in our busy lives, we frequently forget to drink the nutritive herbs essential for the health of our bodies. These beverages are distinguished from caffeinated teas which are made from *M. oleifera* as well as from decaffeinated tea, in which the caffeine has been removed. It is important to understand that there is a huge variety of herbal teas available in the market, each of which is designed to have a specific therapeutic or medicinal benefit. Benefits of herbal tea are as follows:

- Give more calm and relaxed state of mind
- Support heart health
- Aiding with stomach and digestive problems
- Provide cleansing properties of the body
- Promote energy level

- Nourishment of nervous system
- Give strength to immune system
- Provide antioxidants to the body
- Relief from stress
- Stimulating the internal organs
- Promote a good sleep because caffeine free

Ingredients used in formulation of herbal tea

M. oleifera is obtained from the leaves, seeds, flowers, and bark. It shows antioxidant, antiulcer, antiasthmatic, antimicrobial, and anti-inflammatory.[10] Ginger has properties such as antihypertensive, in cold, in beverage, and reduces cholesterol level and blood sugar level.[11] Black pepper is also used as antioxidant, boosts digestion, and aids in weight loss.[11] Cardamom is used as flavoring agent, carminative, and expectorant.[11] Turmeric has active chemical constituents such as curcuminoids, sesquiterpenes, turmerone, and borneol and it shows anti-inflammatory, antifungal, and antidiabetic.[12] Fennel contains volatile oil, ketones, proteins, and bicyclic monoterpene which has diuretic, carminative, flavoring agent, antipyretic, antimicrobial, and anti-inflammatory.[11] Jaggery is natural sweetener, improves digestion, increases energy level, is antioxidant, anticarcinogenic, increases immunity, etc.[13,14] [Figure 1].

MATERIALS AND METHODS

The plants were selected according to their medical use from Borlai farm, Pardi, Valsad - 396 125, Gujarat. Crude drugs such as ginger, black pepper, jaggery, fennel, turmeric, and cardamom are to be used to prepare herbal tea and purchased from the local market.

Different instruments were used for the preparation and evaluation of herbal tea such as incubator, hot air oven, pH meter, refrigerator, electronic balance, autoclave, and muffle furnace marketed by Eie Instruments Pvt. Ltd.

Method of preparation of extract[15]

The extract was made using the decoction method. In different herbal-medicine systems, decoction is a process of extraction that involves boiling the herbal or plant material to dissolve the active chemical components of the substance [Figures 2 and 3] [Table 2].

Evaluations

Organoleptic test

In the organoleptic test, the color, odor, taste, and texture were evaluated by visual inspection of the product.

Physical parameters[16]

- Determination of ash value: Ash value is helpful in determining the quality and purity of crude drug, especially in powder form. The objective of ash vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination.
- Total ash value: Weight accurately about 2 g of powdered drug in a tarred silica crucible. Incinerated at temperature not exceeding 450°C for 4 h, until free from carbon, cooled, and weighted.

% *Total Ash value* = (*wt. of total ash/wt. of crude drug*) × 100

- Water-soluble ash value: The ash boiled with 25 mL of water, filtered, and collected the insoluble matter on an ash less filter paper, washed with hot water, and ignited in a tarred crucible at temperature not exceeding 450°C for 4 h cooled in desiccators, weighted, and subtracted weight off insoluble matter from the total weight of ash.

% *water-soluble ash value* = (*wt. of total ash−wt. of water-insoluble ash/wt. of crude drug*) × 100

- Bulk density and tapped density: Bulk density can be identified as the volume occupied by the

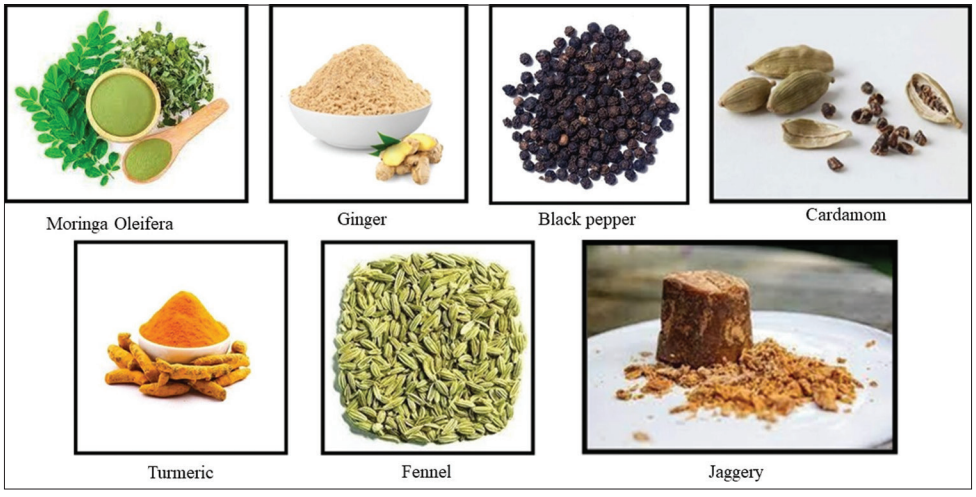


Figure 1: Ingredients used in herbal tea

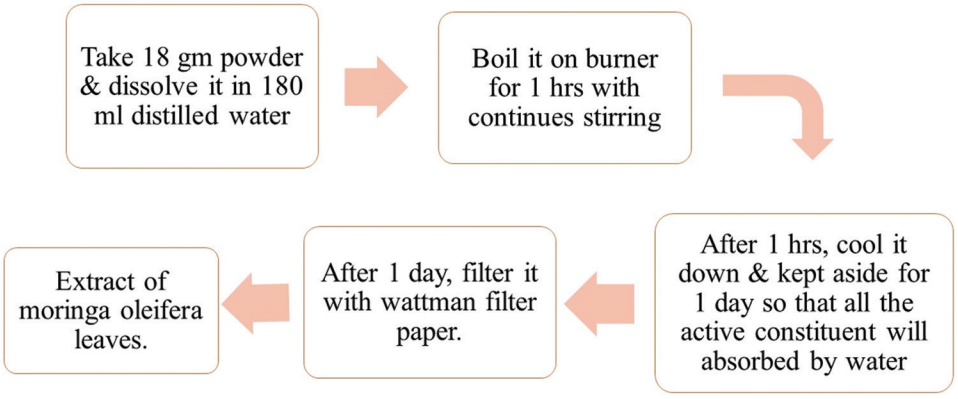


Figure 2: Preparation of extract of *Moringa*

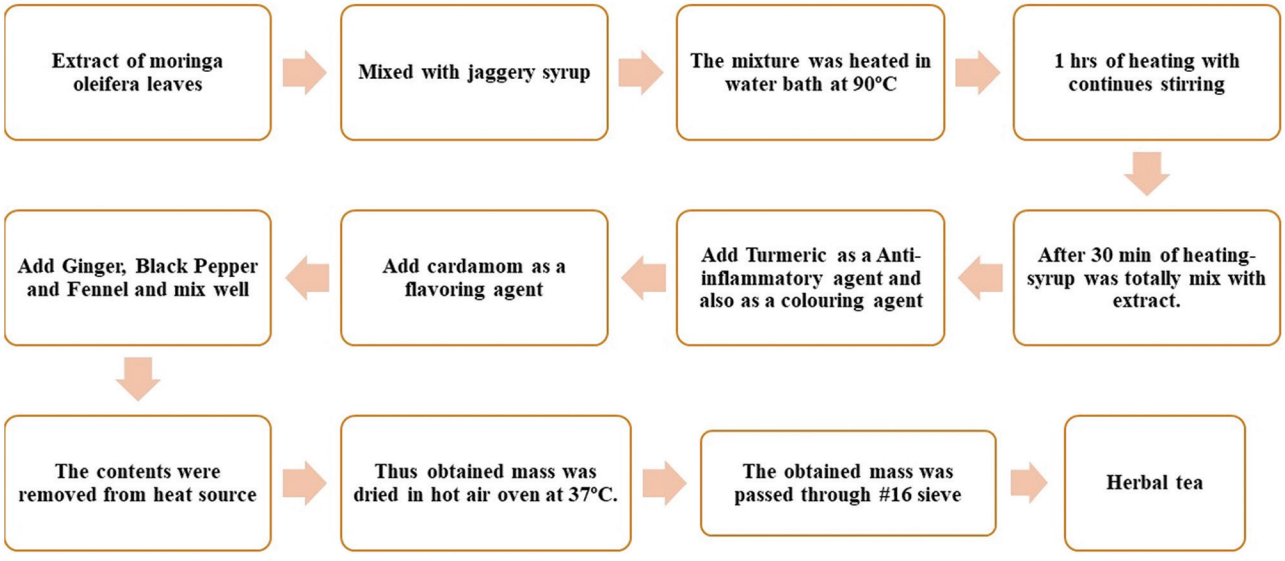


Figure 3: Preparation of herbal tea

solid plus the volume of voids when divided into powder. Whereas, tap density is a different type of bulk density obtained by tapping or vibrating the container in a particular method to achieve more effective particle parking and therefore, it is usually higher than bulk density.

Bulk density = Weight of the powder/ Volume of the packing

Tapped bulk density = Weight of the powder/Volume of the tapped packing

- Carr index and Hausner ratio: They are used in describing the flowability of powder. Carr's index can be determined as the ratio of the difference of the tapped and the bulk densities to the tapped density. According to Carr (1965), who introduced the flowability index, an excellent flowability is between the Carr index of 5–15% while Carr index of above 25% normally shows poor flowability.

Carr's index = (*Tapped density-bulk density/ bulk density*) × 100

Hausner's ratio = Tapped density/Bulk density

- Angle of repose: It was determined using funnel method. In a funnel, the accurately weighed powder was taken. The funnel height was arranged in a manner that the funnel tip just touches the "apex of the heap" or "head of blend." Through the funnel, "the drug excipient blend" was allowed to flow freely on to the surface. Table 3 shows the relationship between angle of repose and powder flow.[17]

Height of pile (cm) = *Average radius of circle in cm Angle of repose* (ɵ) = tan^{-1} (h/r)

- Loss on drying: Take a clean dry petri dish and weigh it. Weigh 2 g of sample powder and transfer to petri dish and weight. Place the Petri dish in a tray dryer and weigh it every 5 min. Allow it to dry until it is constant

Table 3: Preliminary phytochemical analysis[18]

S. No.	Test	Procedure	Observation
1.	Lead acetate test	1 mL filtrate+3 drop of lead acetate solution.	A creamy white precipitate is observed.
2.	Salkowski test	Extract 5 mL+2 mL chloroform+3 mL concentrated sulfuric acid.	A reddish-brown coloration of the chloroform layer.
3.	Dragendroff"s test	1 mL Dragendroff"s reagent+2 mL extract.	Reddish brown precipitate was formed.
4.	Meyer's test	2 mL extract+1 mL of Meyer's reagent.	Precipitates were observed.
5.	Fehling's test	1 mL Fehling A solution+1 mL Fehling B solution, boil it for 5–6 min+2 mL of extract, and heat it in water bath.	Brick red precipitates are observed at the bottom.
6.	Keller–Kiliani test	1 mL filtrate+1.5 mL glacial acetic acid+1 drop of ferric chloride+concentrated H2SO4	Reddish-brown color at the junction of two liquids and upper layer is observed blue in color.

Table 4: pH of the brew on storage in refrigerator

Time (days)	pH of brew solution
1	6.72±0.14
7	6.26±0.32
14	6.57±0.21
21	6.62±0.19
28	6.68±0.39

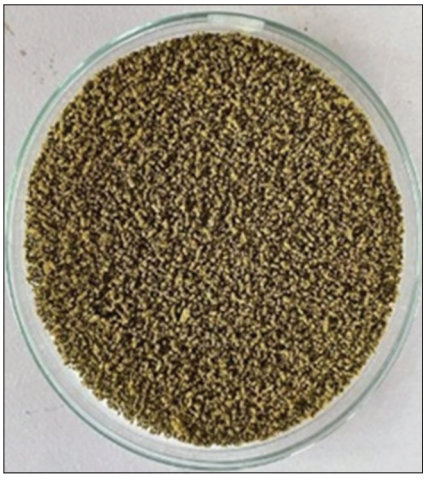


Figure 4: Formulated herbal tea

weight then down the constant dry weight. Calculate the percentage loss on drying and moisture content for the sample.

$$L.O.D = (\textit{weight of sample before drying} - \textit{weight of sample after drying}) \times 100$$

- Dust leak test: Take a 2 g sachet of herbal tea and record its initial weight. Put it in the friability tester for 4 min at 25 rpm. After 4 min record its final weight. The loss of powder should not be more the 1%. Repeat this experiment with 2 more sachets and take the average.

$$(\textit{Initial wt.} - \textit{Final wt./Initial wt.}) \times 100$$

Chemical test

pH test

A tea bag containing the herbal tea was brewed using 200 mL boiling water. At room temperature, the brew was allowed to cool. The digital pH meter was used to determine the pH of the brew solution [Figure 5]. We were testing the pH of the brew solution after it had been stored at 0°C in the refrigerator. This process was carried out every day for a month [Table 4].

Antimicrobial test

The cup and plate method was used to examine the antimicrobial properties of herbal tea and *M. oleifera* leaf extract [Table 5]. A standard Kanamycin 1% solution dissolved in distilled water and a blank as a control were taken, respectively, to test its antibacterial properties.[19,20]

RESULTS AND DISCUSSION

In the first trail batch, the resin was used but the mixing was improper and bad odor was observed after 1 day. As a result, fennel was used in the formulation in place of resin, and proper mixing and odor were noted. Fennel was found to be a good flavoring component in the herbal tea, therefore, we continued our research utilizing F2 batch as the final batch of herbal tea that was made.

Organoleptic test

Organoleptic study is basic study to identify and evaluate the quality of the product. Prepared herbal tea has reported the following parameters[21] [Figure 4].
Color: Greenish yellow
Odor: Pleasant smell
Taste: Sweet-bitter type
Texture: Gritty texture

Chemical test

pH test

pH of the brew of herbal tea was found to be 6.83, slightly acidic brew solution. Stability of the herbal

tea was represented by its pH value. There was no change in taste color and odor of the brew solution so it shows that there is no oxidation reaction took place and prepared herbal tea brew stable on storage. The typical brew pH is correlated with the relative phytochemical composition of the herbs which can identify the quality of the herbal tea.[22]

Figure 5: pH test

Phytochemical test

Phytochemical constituents such as tannin, steroids, flavonoids, alkaloids, carbohydrates, and terpenoids were present in the prepared herbal tea formulations. For the phytochemical constituent screening, following test was performed.[23]

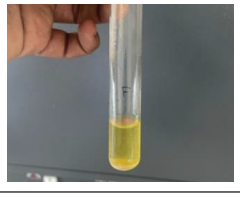
Physical parameters

An ash value indicates the inorganic salts that are either naturally present or that have been added from other sources. To confirm the purity of herbal tea formulation, ash value was frequently utilized. The ash value must be determined to check the presence of little calcium oxalate, silica, and other impurities coming from the soil source. The total ash value is utilized to ensure their purity. The ash of most plant materials contains calcium carbonate as its major component. Calcium carbonate constitutes 25–45% of the ash.[21] Other components include potash (≈10%), iron, manganese, zinc, copper, and heavy metals. Unusually high ash value may reflect contamination or adulteration. Following is the physical test which was performed to check the quality of formulation and found

Table 5: Concentrations for the microbial assay

S. No.	Species of bacteria	Leaf extract	Herbal tea	Standard (kanamycin)	Control
1.	*Bacillus subtilis*	1%	1%	1%	-
2.	*Bacillota cereus*	1%	1%	1%	-
3.	*Staphylococcus aureus*	1%	1%	1%	-
4.	*Escherichia coli*	1%	1%	1%	-

Table 6: Evaluation of phytochemical tests

Phytochemical test	Name of test	Inferences	Figures
Tannins	Lead acetate test	Tannin is present	
Steroids	Salkowski test	Steroid is present	
Flavonoids	Lead acetate test	Flavonoid is present	

(Contd...)

Table 6: *(Continued)*

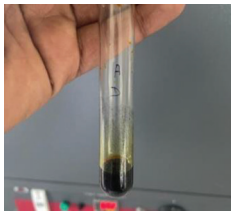
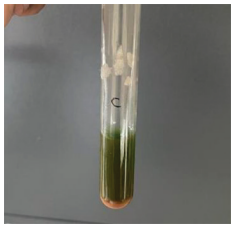
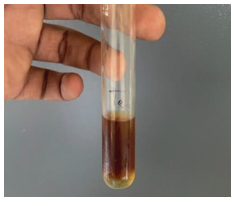
Phytochemical test	Name of test	Inferences	Figures
Alkaloids	Meyer's test	Alkaloid test	
	Dragendroff's test	Alkaloid test	
Carbohydrates	Fehling's test	Carbohydrates are present	
Terpenoids	Salkowski's test	Terpenoid is present	

Table 7: Evaluation of physical parameter

Parameters	Result	Figure of the herbal tea parameters
Total ash value	7.5% w/w	 Total ash value
Water-soluble ash value	2.5% w/w	 Water-soluble ash value

(Contd...)

Table 7: *(Continued)*

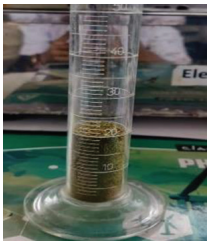
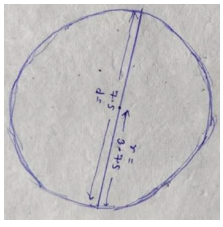
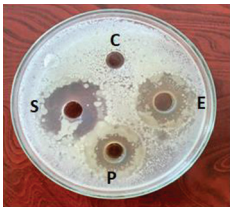
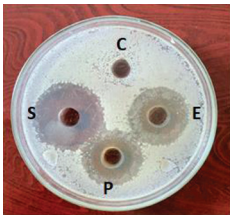
Parameters	Result	Figure of the herbal tea parameters
Bulk density	0.45 g/mL	 Bulk density
Tapped density	0.5 g/mL	 Tapped density
Angle of repose	15.2°	 Angle of repose
Loss on drying	0.5% w/w	 Loss on drying

Table 8: Evaluation of antimicrobial test

Name of bacteria	Zone of inhibition of kanamycin	Zone of inhibition of extract	Zone of inhibition of product	Result
Staphylococcus aureus	22 mm	18 mm	21 mm	 Zone of inhibition
Bacillus Subtilis	34 mm	23 mm	21 mm	 Zone of inhibition

that all the physical parameters of the herbal tea were within the range[24] [Tables 6 and 7].

Bulk density and tapped density identify the flow property of the formulation. Flow property is very crucial parameter to identify flow ability of the formulation. Cohesion force between the particulates was identified by the evaluation of flow parameters. Based on the result of this physical test, the herbal tea granules had an excellent flow.[25]

Anti-microbial assay

The antimicrobial test was carried out on four types of bacteria *Staphylococcus aureus* and *Bacillus subtilis* from which satisfactory result was shown in the following two bacteria's species, *S. aureus,* and *B. subtilis* [Table 8]. We can conclude that the herbal has good antimicrobial property against *S. aureus* and *B. subtilis* by examining the zone of inhibition and comparing it with standard.[19,20]

CONCLUSION

From this study, it shows prepared herbal tea good for health and acceptable to drink. It contains all secondary metabolites. All parameters such as physical and chemical parameters were evaluated successfully. It is a stable formulation which is checked by the pH determination powder characteristics and shows good flow ability so good for leaking test. Herbal tea also examined the microbial growth and its shows potent antimicrobial activity.

ACKNOWLEDGMENT

This research work is supported by ROFEL Shri G. M. Bilakhia College of Pharmacy, Vapi and providing the facilities for evaluation of prepared formulation.

REFERENCES

1. Poswal FS, Russell G, Mackonochie M, MacLennan E, Adukwu EC, Rolfe V. Herbal teas and their health benefits: A scoping review. Plant Foods Hum Nutr 2019;74:266-76.
2. Sagona WC, Chirwa PW, Sajidu SM. The miracle mix of *Moringa*: Status of *Moringa* research and development in Malawi. S Afr J Bot 2020;129:138-45.
3. Abd Rani NZ, Husain K, Kumolosasi E. *Moringa* genus: A review of phytochemistry and pharmacology. Front Pharmacol 2018;9:108.
4. Boukandoul S, Casal S, Zaidi F. The potential of some *Moringa* species for seed oil production. Agriculture 2018;8:150.
5. Builders, PF. Introductory Chapter: Introduction to Herbal Medicine. London: Intechopen; 2019.
6. McKay DL, Blumberg JB. The role of tea in human health: An update. J Am Coll Nutr 2002;21:1-13.
7. Byeon JO, Han JS. A study on perception and actual status of utilization for green tea. Korean J Food Cult 2004;19:184-92.
8. Cohen PA, Ernst E. Safety of herbal supplements: A guide for cardiologists. Cardiovasc Ther 2010;28:246-53.
9. Ravikumar C. Review on herbal teas. J Pharm Sci Res 2014;6:236.
10. Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. *Moringa oleifera* seeds and oil: Characteristics and uses for human health. Int J Mol Sci 2016;17:2141.
11. Kokate CK, Purohit AP, Gokhale DS. Pharmacognosy. Pune: Nirali Prakashan; 2008.
12. Rathaur P, Raja W, Ramteke PW, John SA. Turmeric: The golden spice of life. Int J Pharm Sci Res 2012;3:1987-94.
13. Mahata G. Potentiality of sugarcane juice & jaggery for immunity and employment generation in COVID-19 pandemic situation. Int J Agric Biotechnol Food Sci. 2020;1:25-28.
14. Hirpara P, Thakare N, Kele VD, Patel D. Jaggery: A natural sweetener. J Pharmacogn Phytochem 2020;9:3145-8.
15. Vasudeo K, Pramod K. Biosynthesis of nickel nanoparticles using leaf extract of coriander. Biotechnol Ind J 2016;12:1-6.
16. Bhandare SL, Borkar SP. Formulation and evaluation of immune boosting herbal tea. J Pharmacogn Phytochem 2019;8:3529-35.
17. Singh H, Arora S, Mani M, Mahaur KK, Chandra P. Development of multicomponent formulation of herbal drugs for evaluation of Antidiabetic activity. Pharm Lett 2014;6:219-23.
18. Virshette SJ, Patil MK, Shaikh JR. A review on pharmacological properties and phytoconstituents of indigenous carminative agents. J Pharmacogn Phytochem 2020;9:142-5.
19. Builders PF, Mohammed BB, Sule YZ. Preparation and characterization of a poly-herbal tea with effective antioxidant properties. Sci World J 2020;15:29-34.
20. Jabeen R, Shahid M, Jamil A, Ashraf M. Microscopic evaluation of the antimicrobial activity of seed extracts of *Moringa oleifera*. Pak J Bot 2008;40:1349-58.
21. Alam F, Najum us Saqib Q. Pharmacognostic standardization and preliminary phytochemical studies of *Gaultheria trichophylla*. Pharm Biol 2015;53:1711-8.
22. Choi et al. 2000; Friedman and Jürgens. pH; 2000. Available from: https://www.healthline.com/health/food-nutrition/is-tea-acidic
23. Khandelwal KR. Practical Pharmacognosy. Pune: Pragati Books Pvt Ltd.; 2008.
24. Rao KS, Haran RH, Rajpoot VS. Value addition: A novel strategy for quality enhancement of medicinal and aromatic plants. J Appl Res Med Aromat Plants 2022;31:100415.
25. Garg V, Mallick SS, Garcia-Trinanes P, Berry RJ. An investigation into the flowability of fine powders used in pharmaceutical industries. Powder Technol 2018;336:375-82.