International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
Received: 04-09-2019; Accepted: 06-10-2019
www.pharmacyjournal.in
Volume 4; Issue 6; November 2019; Page No. 29-33

Formulation, evaluation and antibacterial effects of herbal jelly containing miswak stem and betel leaf extracts

Vashudha Dixit[1], Azad Raeen[2], Dr. Alka Saxena[3*]
[1-2] G.C.R.G. Memorial Trust's Group of Institutions, Faculty of Engineering, Lucknow, Uttar Pradesh, India
[3] R&D Department, Acube Life Sciences, Lucknow, Uttar Pradesh, India

Abstract
Medicinal plants are considered to be rich source of therapeutic agents for prevention of diseases. Betel and Miswak are medicinal plants which are being used in Indian system of medicine for their effect on various diseases. The extracts of Miswak and Betel were tested against different bacteria and moulded to form as a unit dosage in the form of jelly. The aim of the study is to observe the antibacterial activity of extracts against different bacterial strains and the formulation and evaluation of the herbal jelly. The quality of the herbal jelly was evaluated with different parameters, physical appearance (colour, odour, taste, texture and shape), pH, average weight, and total microbial count.

Keywords: herbal jelly, formulation, *Salvadora persica*, *Piper betle*

Introduction
Herbs are remedial agents which are created by nature for curing human illness. Traditional medicines are created from medicinal plants which are used by about 80% of the world's population [1]. The herbal formulations are preferred due to lesser side effects and low cost [2]. Herbal drugs assimilate a prevalance of all the officially recognized systems of health in India particularly Ayurveda, Siddha, Homeopathy and Naturopathy [3]. Herbal extracts have been used since ancient times in traditional medicine [4].
Miswak *(Salvadora persica)* is considered to be a medicinal herbal plant [5]. It contains salvadorine and trimethylamine, which are shown to exhibit anti-bacterial effects on cariogenic bacteria such as *Streptococcus mutans* [6]. Miswak is suitable for cleansing teeth, comparatively cheap, possesses various medicinal properties and is easily available in rural areas of developing countries. The Miswak extract has also found its way into the dentifrices in the recent years as anti-plaque and anti-gingivitis agents [7]. It is believed that chewing on these stems facilitate salivary secretions which possibly help in oral cleaning and control of plaque. The oil extracted from this plant is known to exert biological activity and is used to cure gall bladder disease, polio, intestinal worm, gonorrhea, and rheumatic joint pain [8].
Betel (*Piper betle*) leaf are widely used as a mouth freshner after meal. Betel leaf has been described from ancient times as an aromatic, stimulo-carminative, astringent and aphrodisiac [9]. Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, swelling of gum, rheumatism, cuts and injuries [10]. The leaves are full of vitamins like vitamin C, thiamine, niacin, riboflavin, carotene and are a great source of calcium [11]. The leaf has a significant antimicrobial activity against broad spectrum of micro-organisms including *Streptococcus pyrogen*, *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa* etc [12]. Beside these the leaf extract also poses the bactericidal activity against the urinary tract pathogenic bacteria such as *Enterococococcus faecalis*, *Citrobacter koseri*, *Citrobacter fruendi*, *Klebsiella pnemoniae* etc [13].
The objective of this study is to study the phytochemical and anti-microbial properties of miswak stem and betel leaf extracts and formulation and evaluation of the herbal jelly formulated for the various physio-chemical parameters.

Materials and methods
Collection of plant material
The stem of Miswak and Betel leaf were collected from local market of Lucknow. It was authenticated by Acube Lifesciences, Lucknow.

Extraction Procedure
The miswak stem and Betel leaf were cleaned, shade dried and powdered mechanically and stored in air tight containers. The extraction was carried out by maceration. About 5gm of powder was extracted with 20ml distilled water. The extract was kept on rotatory shaker for 48 – 72 hours and after that it was filtered. This crude extract was then stored in refrigerator at 4°C.

Chemicals
All the chemicals used in this study are Analytical Reagent grade of Hi Media Laboratories, and purified according to the standard procedures.

Micro-organisms
For the present study, the microbial strains were provided from Acube Life Sciences, Lucknow, India. The microbial strains comprised of *Staphylococcus aureus*, *E.coli* and *Salmonella sp*.

Phytochemical screening
Preliminary phytochemical analysis includes the tests for the

presence of carbohydrates, proteins, alkaloids, tannins, saponins, fats and flavonoids in the prepared extracts by following standard procedures. The tests followed for the detection of compounds are explained below.

Test for Carbohydrates

- **Fehling test:** 1 ml of sample (extract) was added with equal quantity of Fehling's solutions A and B, slightly heated. The red colored ppt. appeared.

Test for Alkaloids

- **Wagner's reagent test:** Sample (extract) was treated with wagner's reagent (Iodine in potassium iodide). Reddish brown ppt. appeared which indicates the presence of alkaloids.

Test for Tannin and polyphenols

- **Ferric chloride Test:** 1 ml of sample (extract) was treated with 5% freshly prepared ferric chloride solution, deep blue colour came out.

Test for Flavonoids

- **Aqueous sodium hydroxide test:** 10 gm NaOH dissolved in 1L water. 1 ml of sample was treated with this aqueous solution of NaOH, yellow colour appeared.

Test for Saponins

- 5ml distilled water was added to 1ml plant extract and then shaken well, froth formation took place. Stability of froth confirms the presence of saponin in plant extract.

Test for Proteins

- **Biuret Test:** 1% $CuSo_4$ and of 1% NaOH was added to the sample (extract). Appearance of purple colour confirms the presence of protein.

Test for Starch

- Iodine was added to the sample (extract). Appearance of blue or black colour confirms the presence of starch in plant extract.

Test for Fat

- 1ml of distilled water and few drops of ethanol were added to the sample (extract). The white colour precipitate formed showed the presence of fat in the plant extract.

Antimicrobial assay

The antibacterial activity of the extracts were tested using agar well diffusion method. Adequate amount of Nutrient Agar were dispensed into sterile plates and allowed to solidify under aseptic conditions. The test organisms were inoculated with a sterile spreader on the surface of solid medium in plates. The wells were prepared using sterile tip, and a volume (50µl, 75µl and 100µl) of the extract solution at desired concentration was introduced into the well. The bacterial plates were incubated at 37^0C for 24 hours. After incubation all the plates were observed for zones of inhibition. The diameters of zone of inhibition produced by the agent were measured in millimetres and compared with those produced by the commercial antibiotic ciprofloxacin, used as positive control.

Formulation of herbal jelly

25gm of gelatine and 37.5 ml of distilled water were heated separately on water bath and 37.5 ml of glycerine was added to it and heated. Further, 10gm sucrose was added. To clear solution, about 10 ml of extract was added and heating stopped. Lastly, peppermint water (2%) was added as flavouring agent. Citric acid (1%) was added to the above mixture act as a preservative to the above mixture. Then, mixture was poured into moulds and allowed to cool at room temperature. The final herbal jelly was then subjected for evaluation.

Evaluation of herbal jelly

The evaluation of the formulated herbal jelly was carried out for various physical parameters such as appearance, colour, odour, taste, texture, shape, average weight and pH.

a. **Physical Evaluation:** The prepared jelly was inspected visually for clarity and presence of any particle. The jellies were evaluated for colour, odour and taste.
b. **Measurement of pH:** The pH of all the jelly was determined using digital pH meter. 0.5 g of the weighed formulation was dispersed in 50 ml of distilled water and the pH was noted.
c. **Average weight:** To find out weight variation, 10 tablets of formulation were weighed individually using an electronic balance, average weight was calculated and individual tablet weight was then compared with average value to find the deviation in weight.
d. **Total Microbial Counts of Prepared Jelly**
   The herbal preparations are more prone to microbial growth. Hence the jellies formulated were evaluated for total microbial count. The bacteria were evaluated by plate count method as total microbial count.

Fig 1: MIC of Betel against *S.aureus*

Fig 2: MIC of Betel against *E. coli*

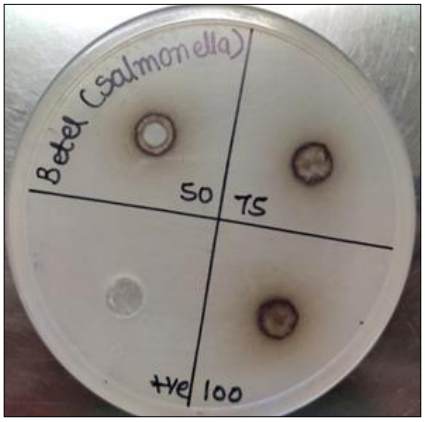


Fig 3: MIC of Betel against *Salmonella*

Fig 4: MIC of Miswak against *E. coli*

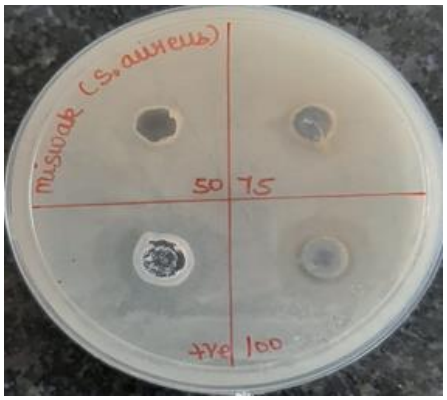


Fig 5: MIC of Miswak against *S. aureus*

Fig 6: MIC of Miswak against Salmonella

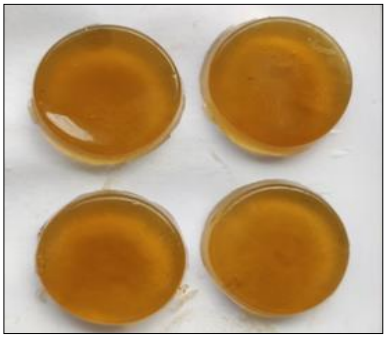


Fig 7: Formulated herbal jellies

Table 1: Result of Phytochemical test of extracts

Phytochemicals	Miswak	Betel
Alkaloid	+	+
Tannin	+	+
Saponin	+	+
Flavonoid	+	+
Proteins	-	+
Carbohydrates	+	+
Starch	+	-
Fat	-	+

Table 2: Result of Physicochemical parameters of developed herbal jelly

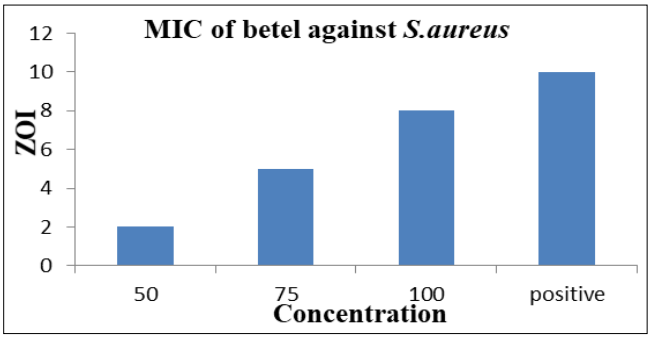
S.No.	Parameter	Observation
1.	Colour	Orange
2.	Odour	Peppermint flavour
3.	Taste	Sweet
4.	Texture	Smooth and transparent
5.	Shape	Round
6.	pH	4.7
7.	Average weight	4.5gms
8.	Microbial Count	Nil

Table 3: The antimicrobial activity and MIC of the prepared Betel extract

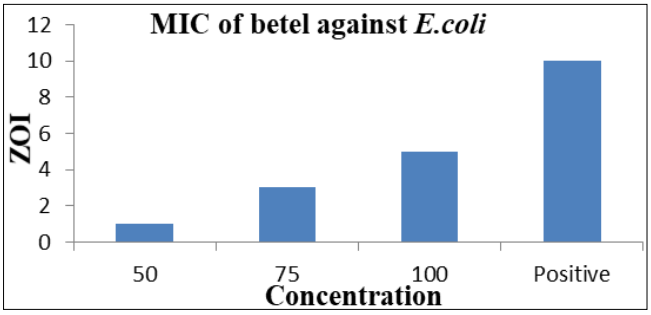
Test Bacteria	Zone of Inhibition (in mm)			
	50 µl	75 µl	100 µl	positive
S. aureus	2	5	8	10
E. coli	1	3	5	10
Salmonella	3	5	8	12

Table 4: The antimicrobial activity and MIC of the prepared Miswak extract

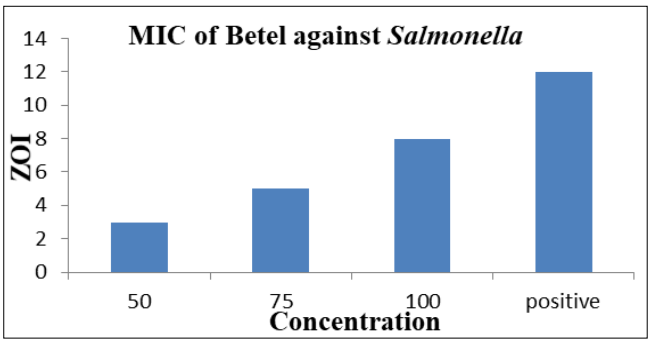
Test Bacteria	Zone of Inhibition (in mm)			
	50 µl	75 µl	100 µl	positive
S. aureus	2	3	4	9
E. coli	3	6	8	20
Salmonella	3	4	6	14



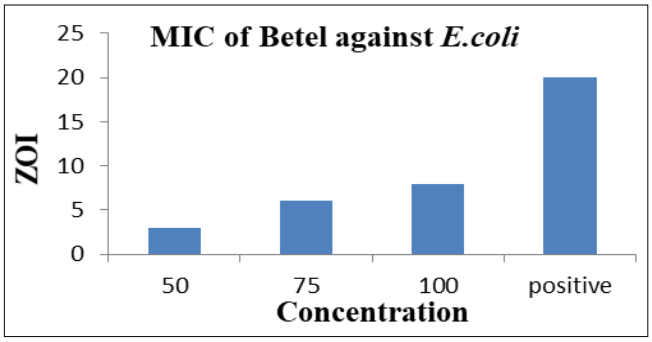
Graph 1: MIC of betel against *S.aureus*



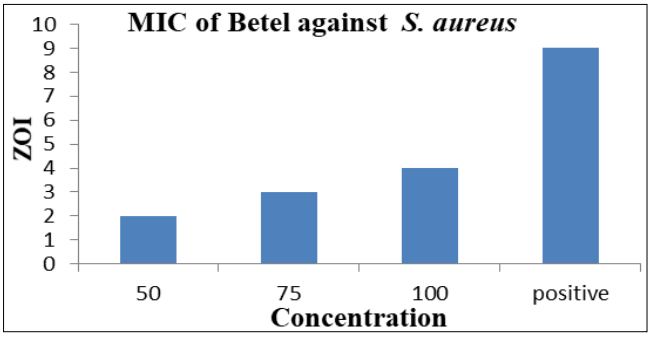
Graph 2: MIC of betel against *E. coli*



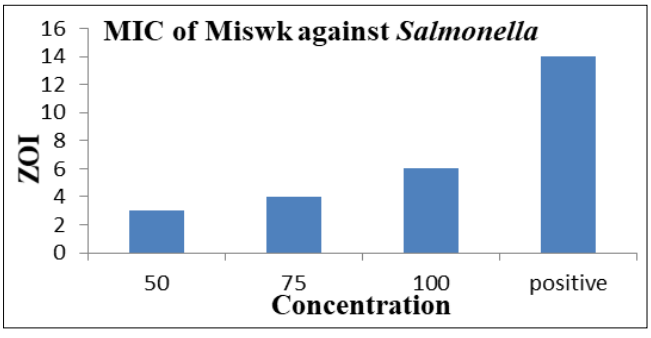
Graph 3: MIC of Betel against *Salmonella*



Graph 4: MIC of Miswak against *E. coli*



Graph 5: MIC of Miswak against *S. aureus*



Graph 6: MIC of Miswak against *Salmonella*

Results and Discussion

In this research, the study was taken up to formulate and develop herbal jelly from extracts of Miswak stem and Betel Leaf. Phytochemical screening of Miswak and Betel extracts revealed the presence of carbohydrates, alkaloids, tannin, saponin, fat, and flavonoids, which are known to be biologically active. These metabolites can exert antimicrobial activity through different mechanisms. The antimicrobial activity and MIC of Betel and Miswak were tested determined by well diffusion method against bacterial strains (*Staphylococcus aureus*, E. coli, *and Salmonella)*, the extract showed zone of inhibition. Herbal jellies were evaluated for various quality control (QC) parameters. The evaluation of formulated herbal jelly was done for various parameters such as colour (Orange), odour (sweet aromatic), taste (sweet), texture (smooth), shape (round), pH(4.7) and average weight (4.5gms). The herbal preparations are more prone to microbial growth. The results indicated that the total microbial count has no colonies found and the formulation was free from microbial contamination.

Conclusion

The present study demonstrates the herbal extracts of Miswak stem and Betel Leaf were successfully formulated in the jelly formulations. The phytochemical properties and antimicrobial activity study of the herbal extracts was observed, the extracts were active against organism *Staphylococcus aureus, Escherichia coli and Salmonella sp*. The prepared jelly formulation was inspected visually for clarity, colour and presence of any particle. The herbal jellies were evaluated for physico-chemical parameters like pH, appearance, colour, texture, flavour, weight variation, and microbial count.

Acknowledgement

Authors are grateful to Acube Life sciences, Lucknow for the guidance, support and providing facilities to carry out this work.

References

1. Londonkar RL, Hugar AL. Physicochemical, phytochemical profiling and Antimicrobial activity of *Pterocarpus marsupium*. International Journal of Pharmaceutical Sciences and Research. 2017; 8:2177-83.
2. Kumar G, Jalaluddin M, Rout P, Mohanty R, Dileep CL. Emerging trends of herbal care in dentistry. J ClinDiagn Res. 2013; 7:1827-9.
3. Udupa N. Status on herbal drugs and their future perspectives. Annals of Phytomedicine. 2016; 5(1):1-3.
4. Kelmanson JE, Jäger AK, van Staden J. Zulu medicinal plants with antibacterial activity. J Ethnopharmacol. 2000; 69:241-6.
5. Almas K, Skaug N, Ahmad I. An *in vitro* antimicrobial comparison of Miswak extract with commercially available non-alcohol mouthrinses. Int J Dent Hyg. 2005; 3:18-24.
6. Al-Bayaty FH, AI-Koubaisi AH, Ali NAW, Abdulla MA. Effect of mouth wash extracted from *Salvadora persica* (Miswak) on dental plaque formation: A clinical trial. J Med Plant Res. 2010; 4:1446-54.
7. Khanra S. Paan Vittik Silpakendra (In Bengali). "Betel Leaf Based Industry". Nabanna Bharati. 1997;

30(2):169.
8. Mariod AA, Matthaus B, Hussein IH. Chemical characterization of seed and antioxidant activity of various parts of *Salvadora persica*. J Am Oil Chem Soc. 2009; 86:857-65.
9. Sudrik S, Fegade S, Shinde M. Anthelmintic activity of *Piper betle* Linn, (Paan/ Nagavalli) aqueous extract. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012; 3(4):467-470.
10. Agarwal T, Singh R, Shukla AD, Waris I, Gujrati A. Comparative analysis of antibacterial activity of four *Piper betel* varieties. Advances in Applied Science Research. 2012; 3(2):698-705.
11. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants, 1956, pp.194. CSIR, New Delhi.
12. Jesonbabu J, Spandana N, Lakshmi KA. *In vitro* antimicrobial potentialities of chloroform extracts of Ethanomedicinal plant against clinically isolated human pathogens. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4(3):624-626.
13. Chakraborty D, Shah B. Antimicrobial, anti-oxidative and anti-hemolytic activity of *Piper betel* leaf extracts. International Journal of Pharmaceutical Technology. 2011; 3(3):192-199.