

Formulation of anti-halitosis mouthwash using aqueous extract of *Mangifera indica* L. kernel based on the evaluation of its antioxidant, antibacterial, and hemolytic activity

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Abstract

Aim: To formulation anti-halitosis mouthwash using aqueous extract of *Mangifera indica* L. kernel based on the antioxidant, antibacterial, and hemolytic activity of the kernel. **Materials and Methods:** Methanolic and aqueous extract of *M. indica* L. kernel were studied for their antioxidant, antimicrobial, and hemolytic properties by hydroxyl free radical scavenging assay, well diffusion method and blood lysis method. Based on the results, mouthwash was formulated from aqueous extract and its antimicrobial and antihalitotic activity was determined by well diffusion method. **Results and Discussions:** The aqueous extract contains the more protein and free phenols than the methanolic extract. Moreover, the aqueous extract showed the higher hydroxyl scavenging activity than the methanolic extract. In antibacterial assay against selected pathogens, it showed following results. Water extract gave better zones for *Bacillus cereus*, *Neisseria meningitides*, *Pseudomonas fluorescense*, and *Bacillus subtilis* than methanolic extract. For anti-halitosis activity study, mouth swabs of five patients suffering from halitosis plated on Muller-Hinton agar and inhibition of growth were examined by measuring the zones. The formulated mouthwash showed good results **Conclusion:** Based on our study, we found that water extract of the kernel possess excellent antioxidant and antibacterial activity, based on which we have formulated and evaluated mouthwash which showed good activity against buccal microflora obtained from halitosis affected patients. Mango kernel can be used as a potent antihalitotic agent in the upcoming days.

Key words: Antioxidant, halitosis, hemolysis, mango kernel, mouthwash

INTRODUCTION

Halitosis or bad breath is the trouble faced by many individuals and can limit their potential to even interact with the society in a positive manner. It has important socio-economic consequences and can reveal important diseases.^[1] It also psychologically affects people due the loss in confidence to speak with others. The prevalence of halitosis has been reported to be as high as 50%. On the other hand, only a few patients visit dentists to seek help for halitosis. This fact suggests that the patients who do visit clinicians may have different psychological characteristics or values concerning their own breath than other individuals.^[2]

There are three types of halitosis such as genuine halitosis, pseudohalitosis, and halitophobia.^[3]

Halitosis can also be termed as bad breath, oral malodor, foetor oris, fetid halitus or stinking mouth.^[4]

Kesar is known as queen of mangoes which is one of the finest varieties of Indian mangoes and is rated to be the best at the home and abroad. The fruits are very attractive, shiny, large sized, and oval^[5] and it has a tiny kernel enclosed in a shell. The kernel weighs about 15 g. The tree grows to a height of 5 m and produces Kesar mango fruit only once a year.

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Received: 14-09-2016

Revised: 15-10-2016

Accepted: 23-10-2016

Large quantities of waste are produced by the agro-fruit industries, in that 45% of the waste is from mango processing industries. The wastes are mango peel and seeds. Research has shown that mango seed kernel contains superior fatty acid contents comparable with cocoa butter.^[6] On the other hand, it contains some amount of proteins and many vitamins such as vitamin A, vitamin E, vitamin K, vitamin B, and vitamin C.^[7] Other than that mango seed kernel have higher antimicrobial activity against various clinically resistant strains including many Gram-negative pathogens.^[8]

There is hardly any study on the anti-halitosis property of mango seed kernel. We have utilized the traditional knowledge and have studied the properties of mango kernel and have formulated an anti-halitotic mouthwash.

MATERIALS AND METHODS

Collection of Mango Kernel

Kesar (*Mangifera indica* L.) variety of mango was purchased from Sivakasi market, and the pulp was separated from the seeds and the seeds were washed under running water. The kernels were separated by breaking the shell and it was shade dried.

Preparation of Extract

The dried kernels were then powdered using mortar and pestle. 20 g of this powder was then added to 40 ml of water and methanol separately and kept under shaking condition for 48 h. These extracts were then filtered, and the extracts were dried in a desiccator. 5 mg/ml concentration of extracts was taken as stock solution for further studies.

Total Crude Protein Estimation

Appropriate dilutions of extract were added to 2 ml of Lowry's reagent and were incubated for 10 min at room temperature. 0.2 ml of Folin–Ciocalteu solution was then added and incubated for 30 min and the absorbance was taken at 660 nm. Bovine serum albumin was taken as standard and the concentration of protein was determined.

Determination of Total Free Phenol

Phenolic groups in a sample decide its antioxidant properties. Appropriate dilutions of samples were mixed with 5 ml of 0.2 N Folin–Ciocalteu reagent and 4 ml of 1 M sodium carbonate. The mixture was allowed to stand for 30 min, and the phenols were determined by calorimetric method at 765 nm. Gallic acid was used as standard.^[9]

Hydroxyl free Radical Scavenging Assay

Hydroxyl scavenging assay of the extract was determined by modifying a standard method. 1.5 ml of different extracts was mixed with 50 μ l of hydrogen peroxide solution. Absorbance was read at 540 nm at different time intervals. Decrease in absorbance indicates increase in scavenging activity. The percentage inhibition was then calculated.^[10]

Antibacterial Assay

Antibacterial activity of the extracts was performed by Kirby-Bauer method. 1-day-old test bacterial strains were inoculated onto Muller-Hinton agar. Using a sterile cork borer, wells were punched, and 100 μ l of extract was added to individual well. The plates were incubated for 24 h at 37°C. After incubation zone of inhibition were recorded.^[11,12]

Formulation of Mouthwash

Mouthwash was formulated using mango kernel extract by modifying the standard protocol.

Ingredients	Composition (%)
Sodium fluoride	0.44
Sorbitol syrup (70%)	15
Methanol	10
Sodium lauryl sulfate	0.4
Saccharin	0.04
Clove oil (Flavor)	0.15
Kernel extract	Make up to 100 ml

Both the extract based mouthwashes were then subjected to sensory analysis, microbial studies using mouth swab from five individual subjects affected by halitosis and certain clinical pathogens.

Hemolytic Activity

About 0.5 ml of sheep red blood cell (RBC) and 0.5 ml of egg yolk were mixed with 1% agar solution at 50°C (pH - 7.3), then poured into sterile Petri plates and wells were bored using a cork borer. 100 μ l of extracts, formulated mouthwash and control mouthwash were pipetted into the well and the plates were incubated at 37°C for 24 h. After 24 h, zones of lysis were recorded.^[13]

RESULTS AND DISCUSSION

Water and methanolic extracts obtained were studied for their protein and phenolic content. The results are shown in Tables 1 and 2.

Table 1: Total crude protein and total free phenols

Type of extract	Total crude protein (mg/ml BSAE)	Total free phenol (mg/ml GAE)
Methanolic extract	2.17	7.22
Aqueous extract	4.09	8.05

BSA: Bovine serum albumin

Table 2: Antimicrobial activity and anti-halitosis activity

Microorganism	Diameter of zone			
	Methanolic extract	Aqueous extract	Mouthwash using aqueous extract	Control (Commercial mothwash)
<i>B. cereus</i>	1.4±0.17	2.1±0.25	1.2±0.12	1.5±0.35
<i>B. subtilis</i>	1.2±0.12	1.9±0.27	1.1±0.25	1.4±0.32
<i>P. fluorescence</i>	1.1±0.46	1.9±0.43	1.0±0.08	1.2±0.48
<i>N. meningitides</i>	1.3±0.47	2.5±0.12	1.3±0.04	1.6±0.10
Mouth swab	-	-	1.3±0.27	1.7±0.44

B. cereus: *Bacillus cereus*, *B. subtilis*: *Bacillus subtilis*, *P. fluorescence*: *Pseudomonas fluorescence*, *N. meningitides*: *Neisseria meningitides*

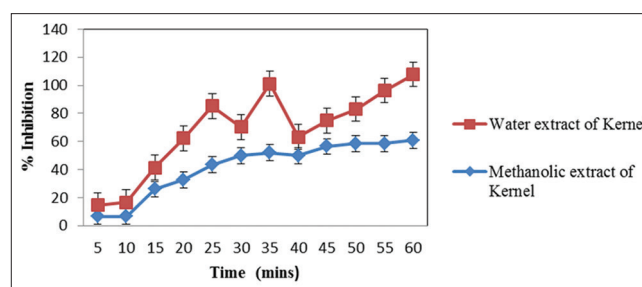
Aqueous extracts contained more protein than the methanolic extract. This may be due to the presence of polar proteins. In the case of free phenolic assay, the results obtained for aqueous extract was more than methanolic extract, which may be due to the presence of dissolved proteins and flavonoids in the extract.

Hydroxyl free radical scavenging assay is an assay used to determine the radical scavenging ability of any extract. The aqueous extract also showed higher scavenging ability throughout the study [Figure 1].

The clinically resistant test bacterial samples were used for this study. Water extract gave better zones for *Bacillus cereus* (2.1 ± 0.25 cm) and *Neisseria meningitides* (2.5 ± 0.12 cm), wherein the methanolic extract gave a zone of 1.4 ± 0.17 and 1.3 ± 0.47 cm, respectively. In the case of *Pseudomonas fluorescence* and *Bacillus subtilis* water extract gave an average zone of 2 cm, whereas methanolic extract gave an average zone of 1.3 cm.

Based on the results obtained, aqueous extract of the kernel was used to prepare mouthwash and its antibacterial activity was studied using the test strains previously discussed. A commercially available mouthwash was used as a control. For anti-halitosis activity study, mouth swabs of five patients suffering from halitosis were subcultured. These were then plated on Muller-Hinton agar, and inhibition of growth was examined by measuring the zones obtained and the diameter of zones was found to be 1.3 ± 0.27 cm, which was less than the control by 0.4 ± 0.14 cm.

Hemolytic activity is used to study the ability of sample to lyse RBCs. Water extracts showed a zone of 0.1 ± 0.024 cm diameter and the methanolic extract showed 0.4 ± 0.092 cm diameter. In the case of the formulated mouthwash, it showed a zone of 0.2 ± 0.054 cm diameter and the control gave 0.2 ± 0.018 cm diameter.

**Figure 1:** Hydroxyl scavenging assay

CONCLUSION

Plant samples have been traditionally used in India for the treatment of buccal issues. Mango kernel has hardly been studied for their activity against halitosis. Based on our study, we found that water extract of the kernel possess excellent antioxidant and antibacterial activity, based on which we have formulated and evaluated mouthwash which showed good activity against buccal microflora obtained from halitosis affected patients. Mango kernel can be used as a potent anti-halitotic agent in the upcoming days.

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Source of Support: Nil. **Conflict of Interest:** None declared.