

Evaluation of Anti-Microbial (In-Vitro) Activity of Amarantha Herbal Mouthwash

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Abstract: Halitosis is one of the complaints encountered in the clinical settings where individual experiences unpleasant odours originating from oral cavity. Oral pathogens play important role in the generation of volatile sulphur compounds (VSC) in oral cavity that result into the development of bad breath. Mouthwashes are widely accepted to deliver antimicrobial agents so as to eliminate VSC producing bacteria and control bad breath. Most commonly used mouthwashes are effective but associated with multiple adverse effects like staining of teeth, gums, tongue and alteration of taste sensation after mouth rinsing. Ari Healthcare Pvt. Ltd. has conceptualized and developed Amarantha Herbal Mouthwash. The present study was conducted to evaluate antimicrobial activity of Amarantha Herbal Mouthwash against oral pathogens like *Staphylococcus aureus*, *E. coli* and *Candida albicans*. Muller Hinton agar was used for *Staphylococcus aureus* & *E. coli* and PDA was used for *Candida albicans* wherein 100µl of cultures of respective microorganisms were spread. All experiments were performed in triplicate. The assessment of the antimicrobial activity was based on the measurement of the diameter of the zone of inhibition. The zone of inhibition for Amarantha Herbal Mouthwash against *Staphylococcus aureus*, *E. coli* and *Candida albicans* was 25mm, 17mm and 13mm respectively which suggests its significant antimicrobial activity against all the 3 microorganisms. This activity was comparable to that of standard Chlorhexidine. It can be concluded that Amarantha Herbal Mouthwash possesses antimicrobial activity against *Staphylococcus aureus*, *E. coli* and *Candida albicans* which further endorses its role in controlling Halitosis.

Keywords: Halitosis, Amarantha Herbal Mouthwash, *Staphylococcus aureus*, *E. coli*, *Candida albicans*

1. Introduction

Halitosis is an oral health condition characterized by unpleasant odours emanating consistently from the oral cavity^[1]. Other terminologies used for halitosis are bad breath, oral malodor, fetor oris, and fetor ex-ore^[2]. Almost every individual awakes with bad breath each morning^[3]. It has been estimated that 8-50% of people in developed world perceive oral bad odor^[4]. The origin of halitosis may be related both to systemic (15%) and oral conditions (85%)^[5]. Accumulation of food residues at the back and in the furrows of the tongue, which are then broken down into volatile sulphur compounds (VSC) and other volatile compounds by bacteria are considered to be the major causes of bad breath^[1]. Inter-dental plaque and gingivitis may also play a fundamental role to cause halitosis^[3].

Though causes for halitosis include both oral and non-oral factors; it was observed that, 76% of 2000 patients in a clinical study had oral factors responsible for Halitosis.^[6] It is also known that dental plaque is primarily responsible for caries, gingivitis and periodontitis^[7] which are considered to be important conditions responsible for halitosis. Various in-vitro and in-vivo studies have also reported that volatile odoriferous substances are produced by putative periodontal pathogens and their products of inflammation formed in oral cavity. Considerable reduction in this microbial count was demonstrated by intervention studies wherein significant reduction in halitosis was achieved suggesting concrete association between oral pathogens and halitosis^[6]. Hence it

is prudent to focus attention on local oral conditions in the management of halitosis.

Dental plaques are conventionally controlled by mechanical methods like tooth brushing and flossing. However these methods require dexterity, time, and motivation. Moreover these methods may still fail to remove all the microbes and plaques from the areas that are difficult to reach. Hence mouthwashes are widely accepted as an adjunct to brushing to deliver active agents to dental and periodontal regions^[8]. Since control of VSC producing bacteria remains the mainstay of treatment along with the plaque control, mouthwashes aim at delivering antimicrobial agents to the oral cavity. Most commonly used mouthwash being Chlorhexidine, which has exhibited antimicrobial activity accompanied by significant reduction in plaques and gingivitis.^[8] However prolonged use of Chlorhexidine is limited because of associated adverse effects like alteration of taste sensation after mouth rinsing, staining of teeth, tongue, gums and development of supragingival calculus in some cases.^[8] Hence there is increased interest in development of alternative safe and effective medicine for management of Halitosis. Considering this, Ari Healthcare Pvt. Ltd. has conceptualized and developed alcohol free Amarantha Herbal Mouthwash for the management of Halitosis.

Amarantha Herbal Mouthwash contains Nimba extract (*Azadirachtaindica*), Triphala extract (Ayurvedic Classical formulation), Gandhapura oil (oil of *Gaultheria fragrantissima*) and Clove oil (oil of *Syzygium aromaticum*).

In Ayurveda, these ingredients are successfully used since thousands of years in the management of oral cavity disorders.^[12-18] Most of the ingredients of mouthwash exhibit anti-inflammatory, antimicrobial and antiseptic activities.^[12-18] Therefore Amarantha Herbal Mouthwash is recommended for effective management of Halitosis, dental plaques, toothaches and gingivitis related pain and inflammation. The present study was conducted to evaluate anti-microbial activity of Amarantha Herbal Mouthwash in comparison with Chlorhexidine and Marketed formulation. This in-vitro study was carried out against oral pathogens i.e *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

2. Materials and Methods

Inoculum preparation:

Test organisms were grown in respective media to get approximately 10^6 cfu per ml and 100 μ l of this was used for the antimicrobial assay

Inoculation of Test Plates:

- Muller Hinton agar (Hi Media) was used for bacterial cultures and PDA was used for fungal culture. The dried surface of a Mueller-Hinton agar plate was inoculated by spreading culture suspension (100 μ l) on agar surface.
- The cups were bored in agar medium spread with the test organism, using a sterile cork borer with 8 mm inner diameter and 4 different mouth wash solutions (100 μ l) were added to the wells in triplicates. Amarantha Herbal Mouthwash was directly used. Marketed formulation and standard Chlorhexidine were used as control. Plates were kept in the freeze for pre-diffusion for 30 minutes and then placed in an incubator set to 37°C for 24 hours for bacteria and 30°C for fungus. All experiments were performed in triplicate.
- The assessment of the antimicrobial activity was based on the measurement of the diameter of the zone of inhibition.
- After incubation, each plate was examined for presence or absence of the antimicrobial activity. If activity was

observed the diameters of the zones of inhibition were measured, including the diameter of the well.

3. Results

Antimicrobial activity of Amarantha Herbal Mouthwash:

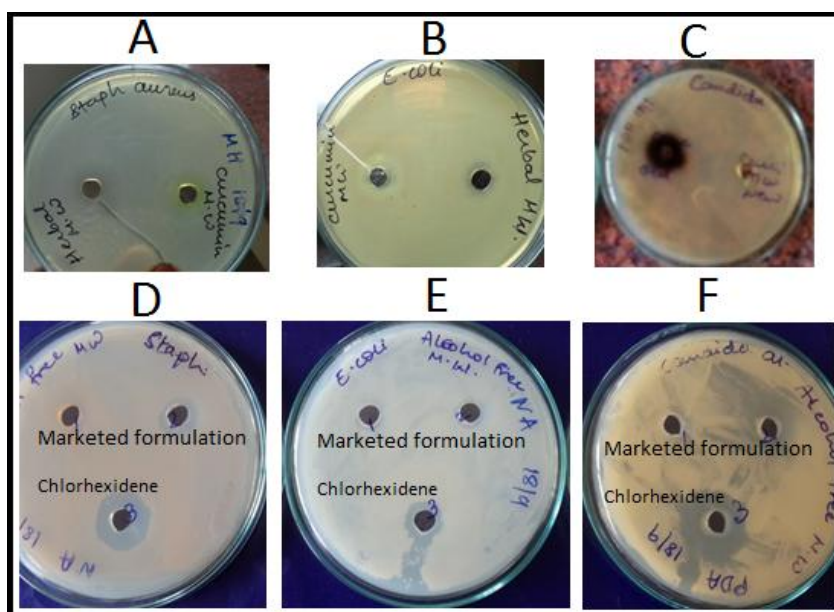
It is evident from the results that, significant antimicrobial activity is demonstrated by Amarantha Herbal Mouthwash against bacteria and fungi. Zone of inhibition of Amarantha Herbal Mouthwash against *E. coli* was 17 mm; which is significantly greater than Marketed Formulation (11) and Chlorhexidine (15). 25mm zone of inhibition was observed with Amarantha Herbal mouthwash against *Staphylococcus aureus*. The Marketed Formulation and Chlorhexidine showed 16mm and 18mm respectively. Amarantha Herbal Mouthwash showed significant inhibition of the test bacteria *E. coli* and *Staphylococcus aureus* compared to the standards. Antifungal activity of mouthwashes was also observed against *Candida albicans*; however Chlorhexidine mouthwash proved superior to other formulations in this regard.

The details are presented in Table 1 and Fig 1.

Table 1: Antimicrobial activity of Amarantha Herbal Mouthwash against *Staphylococcus aureus*, *E. coli* and *Candida albicans*

Name of the organism	Zone of Inhibition (mm)		
	Amarantha Herbal Mouthwash	Marketed Formulation	Chlorhexidine
<i>E. coli</i>	17	11	15
<i>Staphylococcus aureus</i>	25	16	18
<i>Candida albicans</i>	13	13	23

Fig. 1: Antimicrobial activity of Amarantha Herbal Mouthwash (Alcohol free) in comparison with Marketed Formulation and Chlorhexidine against *Staphylococcus aureus*(A&D) and *E. coli* (B&E) and *Candida albicans*(C&F).



4. Discussion

Oral conditions such as halitosis and periodontal diseases have been major issues of concern in the field of dentistry since long time. Various epidemiological studies have also demonstrated association between halitosis/periodontitis causing oral pathogens and increased risk for atherosclerosis, thromboembolic events and several respiratory conditions like nosocomial pneumonia.^[9, 10] Therefore it is necessary to provide timely attention towards apparently trivial looking oral cavity related disorders such as halitosis.

The main etiological factor for development of most forms of periodontal diseases is considered to be the dental plaque, and hence more attention is given to the research in the field of effective anti-plaque agents.^[11] Various chemical agents have been extensively studied in this regard and are shown to possess significant antiplaque and antibacterial activities. The most common examples being chlorhexidine and povidon-iodine mouth washes. However their widespread use is limited owing to the various adverse effects. It is also reported that these have cytotoxic effect on human gingival fibroblasts.^[11] Therefore search for alternative, safe and effective anti-plaque agents is the need of an hour. Ari Healthcare Pvt. Ltd. has conceptualized and developed a polyherbal alcohol free herbal mouthwash for effective management of halitosis, dental plaques and other periodontal diseases.

Amarantha Herbal Mouthwash contains Nimba extract (*Azadirachta indica*)^[12-14], Triphala extract (Ayurvedic classical formulation)^[17-18], Gandhapura oil (oil of *Gaultheria fragrantissima*)^[15] and Clove oil (oil of *Syzygium aromaticum*)^[16].

In Ayurveda, Nimba (*Azadirachta indica*) has been used as *Krimighna* (anti-microbial). It is useful in the management of oral cavity diseases including halitosis, mouth ulcers, periodontal disease and dental plaque.^[12-14] In many research studies it has been observed that the bioactive compounds such as *nimbidin*, *nimbolide*, *gedunin*, and *mahmoodin* have anti-inflammatory, anti-septic and anti-bacterial properties. *Azadirachta indica* is active against *Klebsiella*, *Staphylococcus* and *Serratia* species. It is also active against *Streptococcus mutans* and *Streptococcus faecalis*^[12-14].

Gandhapura oil is a volatile oil obtained from *Gaultheria fragrantissima*. The active ingredient i.e. Methyl salicylate is used in mouthwash. Methyl salicylate is used for gingival inflammation. It is also known to have analgesic activity^[15].

Clove oil is traditionally used in the treatment of various oral cavity diseases. Clove helps to decrease halitosis. Active chemical constituent of clove i.e. *Eugenol* possesses local anaesthetic and antiseptic properties hence it is useful in oral cavity diseases such as gingivitis, dental pain and dental plaque. It reduces the harshness of bad breath caused due to variety of reasons and generates fresh breath after oral application^[16]. Clove oil has an inhibitory action against various organisms like *Streptococcus aureus*, *Monocytogenes*, and *Aspergillus* causing oral cavity diseases such as dental plaque and halitosis. It is also active against gram negative bacteria^[16].

In Ayurveda classical formulation 'Triphala' has been used for oral cavity diseases since thousands of years due to its astringent property. Mouthwash prepared from Triphala has shown significant anti-plaque activity. It is also found useful in reducing gingival inflammation^[17-18].

It is clear from the above discussion that almost all the ingredients of Amarantha Herbal Mouthwash possess antibacterial activity. Most of them have been used in the management of oral cavity disorders traditionally and also have been evaluated experimentally to assess their efficacy. Therefore it was proposed that Amarantha Herbal Mouthwash possesses antibacterial activity against oral pathogens and would effectively manage halitosis and related conditions.

Present in vitro study was conducted to evaluate antibacterial activity of Amarantha Herbal Mouthwash against oral pathogens like *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

It is observed that, Amarantha Herbal Mouthwash exhibits significant antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Its activity against *E. coli* is comparable to chlorhexidine and superior to marketed formulation. Antibacterial activity against *Staphylococcus aureus* was superior to other formulation but antifungal activity as suggested by zone of inhibition against *Candida albicans* was comparatively weaker than chlorhexidine. Hence results suggest that Amarantha Herbal Mouthwash shows significant antibacterial and antifungal activity against *Staphylococcus aureus*, *E. coli* and *Candida albicans*. The antibacterial and antifungal activity of Amarantha Herbal Mouthwash is attributed to the synergistic action of all the ingredients present in it.

5. Conclusion

It can be concluded that Amarantha Herbal Mouthwash possesses antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. It can be used for effective management of Halitosis, dental plaques, toothaches and gingivitis related pain and inflammation.

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