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Efficacy of anti-bacterial and anti-fungal action on four medicinal plants extract the A.arabica, T.chebula, A.indica, and V.vinifera against Streptococcus mutans and Candida albicans -An in-vitro study

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ABSTRACT

The aim of the study was to test the anti-bacterial, and anti-fungal activity of four medical plants extract the A.arabica, T.chebula, A.indica, and V.vinifera against the streptococcus mutants and Candida albicans organisms. The extracts obtained from the medicinal plant of A.arabica, T.chebula, A.indica, and V.vinifera was procured and powdered. The minimum inhibitory concentration of the obtained extracts was determined by using the tube dilution method, and the bacterial strains of Streptococcus mutants and Candida albicans were cultured into the extract obtained at a concentration of 1mg/ml, 500μg/ml, 250μg/ml, and 100μg/ml respectively. The extracts obtained from A.arabica and T.chebula had a better anti-bacterial property when compared to the other two medicinal plants and the extracts obtained from A.indica and V.vinifera had a better anti-fungal property when compared to the other two medicinal plants. These plants extracts showed the anti-bacterial property in previous studies to add on to the review of literature in our study it proved that A.indica and V.vinifera had a better anti-fungal activity when compared to another two medicinal plant extract. The clinical significance of the medicinal plant extract can be effectively used as an anti-bacterial and anti-fungal agent as it was compared with the gold standard values of Streptomycin and Amphotericin B.

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INTRODUCTION

Each civilization that has progressed produced the use of medicinal plants. The recent increase in the popularity of herbals products globally may reflect the fact that a lot of people have disbelief with the current traditional medical practice. Most people feel that herbal medicines are safer and less toxic (Li and Weng, 2017). Popularly used herbal supplements are licorice, ginger, garlic, and clove. Much of the information available herbal supplements and remedies are market/self commentaries driven and not supported by a clinical study. Moreover, the quality, strength, and purity of the medi-

cation depend on the time, place, and season of cultivation apart from the techniques used in processing and packing. Although there may be benefits to using herbal medicines in the practice of dentistry, only a few studies support the effectiveness of the product, most of them remain undocumented at the primary level of clinical research.

The extract of A. Arabica (bark) is used potential to cure oral disease and commonly used in India as a chewing stick as a daily oral hygiene regimen in many communities. The composition of A. Arabica is a mixture of magnesium, calcium, and potassium salts. They are studies that provide antimicrobial and antibacterial properties (Clark *et al.*, 1993). The extract of T. chebula acts as an anti-caries and antibacterial agent (Saxena *et al.*, 2017), and studies have reported that it decreases the microbial count of S.mutans (Nayak *et al.*, 2010). A. indica is an effective extract that has an anti-plaque property and commonly used as a mouthwash (Vanka *et al.*, 2001). Most of the dentifrices combine the herbal extracts with the surfactant lauryl sodium sulfate as a result of the combined effects of the surfactant and the active ingredient on microbial cell walls. V. vinifera is used as an antifungal agent in dentistry (Stempien *et al.*, 2018).

Herbal products like chamomile, clove oil, Echinacea, eucalyptus, fennel, ginger, liquorices root, tincture of myrrh, nettle leaves, tea tree oil, witch hazel, and watercress are often seen in herbal dental products. Herbal products have also been used for the management of halitosis compounds used are Parsley has been known as a breath sweetener and is served after eating food in restaurants. Sometimes a combination of parsley and cottonseed oil is also used in the treatment of halitosis. Licorice is used to flavor herbal toothpaste and mouthwashes for the treatment of halitosis. Products synthetically made from essential oils such as eucalyptol, eugenol, menthol, and phenol. They are disinfectants and are mainly used as topical anesthetics. They are various studies published by other plant extracts since these extracts have not been used to find out the anti-bacterial and anti-fungal property.Hence, the objective of the study was to determine the antibacterial and anti-microbial efficacy of four medicinal plants extract the A.arabica, T.chebula, A.indica, V.vinifera on different concentration (Kumarasamy *et al.*, 2014; Barani *et al.*, 2014; Shireen *et al.*, 2015; Rekha *et al.*, 2014).

MATERIALS AND METHODS

Collection of plant extract

Acacia arabica (Powder), Terminalia chebula (dried fruit powder), Azadirachta indica (Fresh stem), Vitis vinifera (Fresh seeds) were brought from the open market from Chennai. The experiments were carried out at the Department of Microbiology, SRM Dental College, Chennai. The wild strains of streptococcus mutans and Candida albicans were used. The quality control of the organism and sterility control was carried out prior to the start of the study for satisfaction.

Determination of Minimum inhibitory concentration (MIC)

MIC was determined by a micro-dilution method using serially diluted (2 folds) plant extracts, according to the National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 2000). The MIC of the extracts was determined by dilution of Sample A and Sample B of various concentrations of 1mg/ml, 500μg/ml, 250μg/ml, 100μg/ml, respectively. The test tube was taken, and an equal volume of the nutrient broth and each extract of the medical plant were mixed in the test tube. Specifically, 0.1 ml of standardized inoculums ($1X´10^7$CFU/ml) was added in each tube. The tubes were incubated aerobically at 37^{o}C for 24 h. The included antibiotic control was the test tube containing the growth media and extract without the inoculums, and organism control includes the test tube containing saline, growth media with the inoculums. The highest dilution was noticed in the lowest concentration of the extract, which produced no visible bacterial growth (no turbidity) when compared to the control tubes.

RESULTS AND DISCUSSION

Table 1 showed that four samples of different medicinal plant extract were used with the positive control as streptomycin (10μg/ disc**)** and negative control as Dimethyl Sulfoxide and the concentration which were tested are 1000μg, 500μg, 250μg, 100μg/disc, and the target organism was streptococcus mutans

Table 2 showed that four samples of different medicinal plant extract were used with the positive control as amphotericin B (10μg/ disc**)** and negative control as Dimethyl Sulfoxide and the concentration which were tested are 1000μg, 500μg, 250μg, 100μg/disc, and the target organism was Candida albicans.

Figures 1, 2, 3 and 4 showed the antibacterial activity of the four medicinal plants extract the A.Arabica, T.Chebula, A.Indica, and V.Vinifera against streptococcus mutants clockwise from top to bottom in different concentration were tested from 1000μg, 500μg, 250μg, 100μg/disc and solvent of Dimethyl

Table 1: Zone of inhibition seen in different concentrations by inhibiting the micro-organism – streptococcus mutants

S.No	Microorganism	Zone of Inhibition in mm				
		1000 μg	500μg	250μg	100μg	Streptomycin (10μg/ disc)
	Streptococcus mutans					
1.	A. Arabica	18±0.66	15 ± 0.88	11 ± 0.88	9 ± 0.57	21 ± 0.33
2.	T. chebula	19 ± 0.33	15 ± 0.33	10 ± 0.33	8 ± 0.33	22 ± 0.33
3.	A. indica	16 ± 0.88	13 ± 0.66	10 ± 0.66	8 ± 0.66	22 ± 0.33
4.	V. vinifera	18 ± 0.57	15 ± 0.66	10 ± 0.33	9 ± 0.33	19 ± 0.33

Table 2: Zone of inhibition seen in different concentrations by inhibiting the micro-organism- Candida Albicans

S.No	Microorganisms	Zone of Inhibition (in mm)				
		1000 μg	500μg	250μg	100μg	Amphotericin B (10μg/ disc)
	Candida albicans					
1.	A. Arabica	11 ± 0.33	8 ± 0.33	6 ± 0.33	6 ± 0.33	13 ± 0.57
2.	T. chebula	14 ± 0.66	11 ± 0.33	10 ± 0.33	9 ± 0.33	13 ± 0.66
3.	A. indica	17 ± 0.33	14 ± 0.33	11 ± 0.57	10 ± 0.33	16 ± 0.57
4.	V. vinifera	15 ± 0.57	12 ± 0.33	9 ± 0.33	9 ± 0.33	14 ± 0.57

sulfoxide were used with streptomycin(10μg/ disc**)** as control.

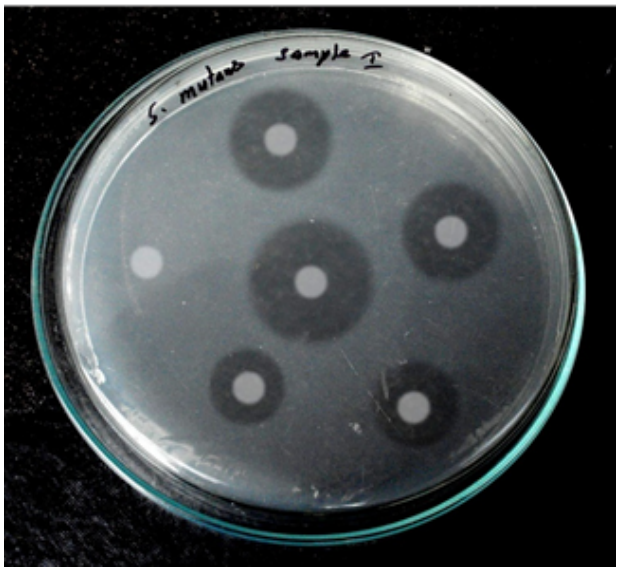

Figure 1: Anti-bacterial activity of extract of A. Arabica against streptococcus mutants clockwise from top:1000, 500, 250, 100 μg/disc and solvent (Dimethyl sulfoxide) control center:streptomycin (10 μg/disc) control

Figures 5, 6, 7 and 8 showed the anti-fungal activity of the four medicinal plants extract the A.Arabica, T.Chebula, A.Indica, and V.Vinifera against candida Albicans clockwise from top to bottom in different concentration were tested from 1000μg, 500μg, 250μg, 100μg/disc and solvent of Dimethyl sulfoxide were used with amphotericin B(10μg/ disc**)** as control.

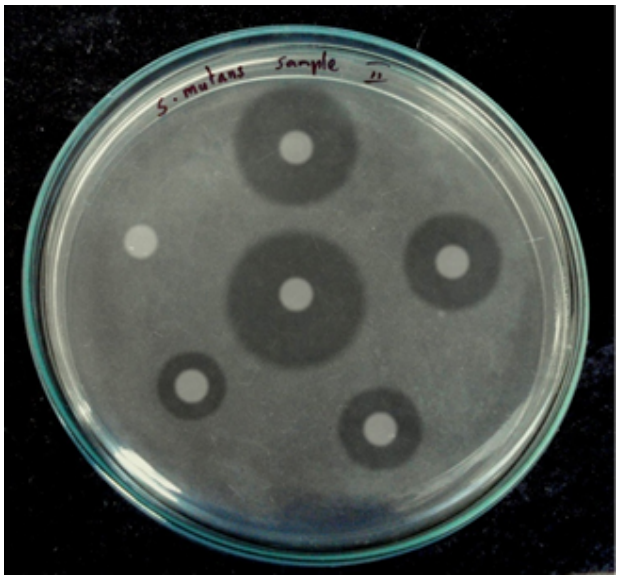

Figure 2: Anti-bacterial activity of extract of T. chebula against streptococcus mutants clockwise from top:1000, 500, 250, 100 μg/disc and solvent (Dimethyl sulfoxide) control center:streptomycin (10 μg/disc) control

Herbal extracts are widely used in various fields of

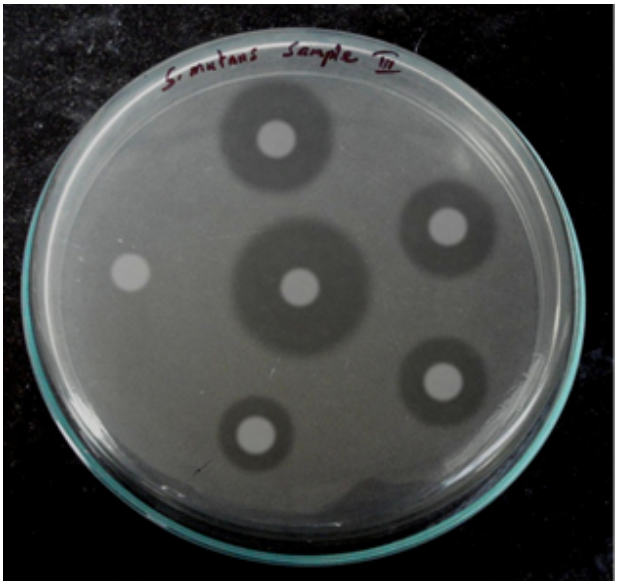

Figure 3: Anti-bacterial activity of extract of A. indica against streptococcus mutants clockwise from top: 1000, 500, 250, 100μg/disc and solvent (Dimethyl sulfoxide) control center: streptomycin (10μg/disc) control

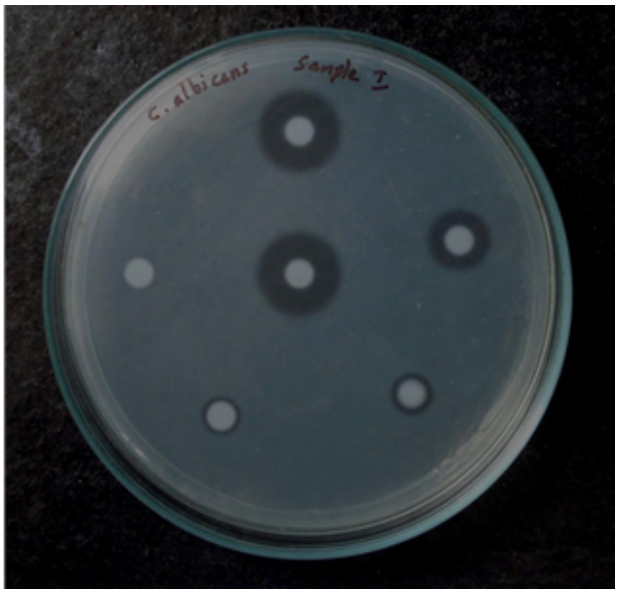

Figure 5: Anti-fungal activity of extract of A.Arabica against candida Albicans clockwise from top: 1000, 500, 250, 100 μg/disc and solvent (Dimethyl sulfoxide) control center: Amphotericin B (10 μg/disc) control

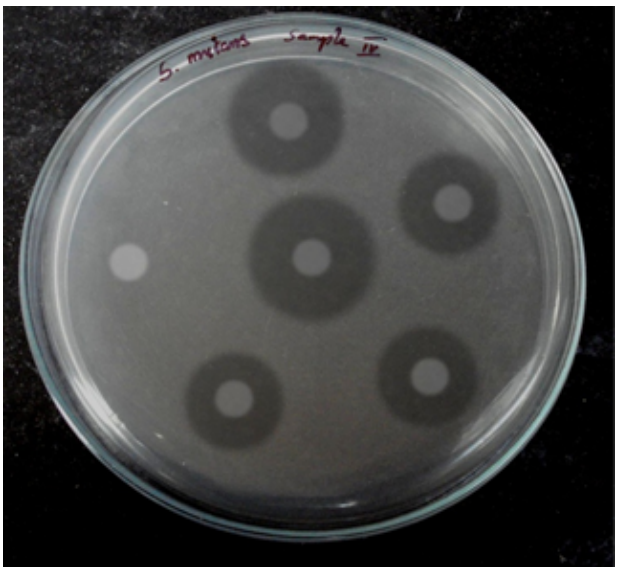

Figure 4: Anti-bacterial activity of extract of V. vinifera against streptococcus mutants clockwise from top:1000, 500, 250, 100 μg/disc and solvent (Dimethyl sulfoxide) control center:streptomycin (10 μg/disc) control

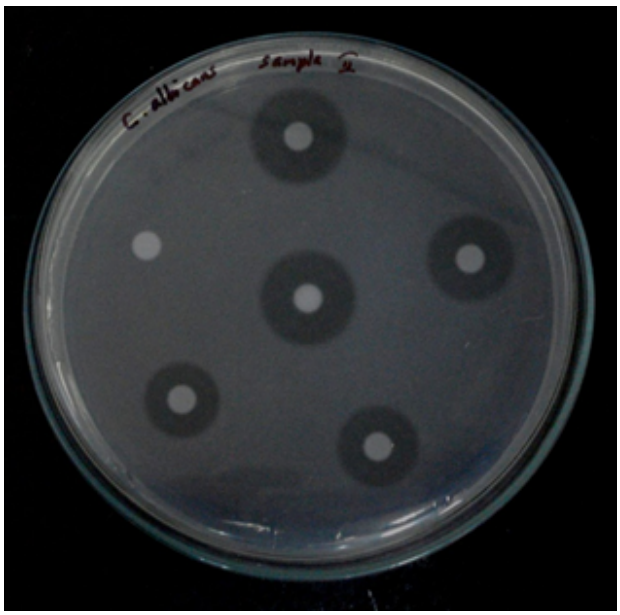

Figure 6: Anti-fungal activity of extract of T. chebula against candida Albicans clockwise from top: 1000, 500, 250, 100 μg/disc and solvent (Dimethyl sulfoxide) control center: Amphotericin B (10 μg/disc) control

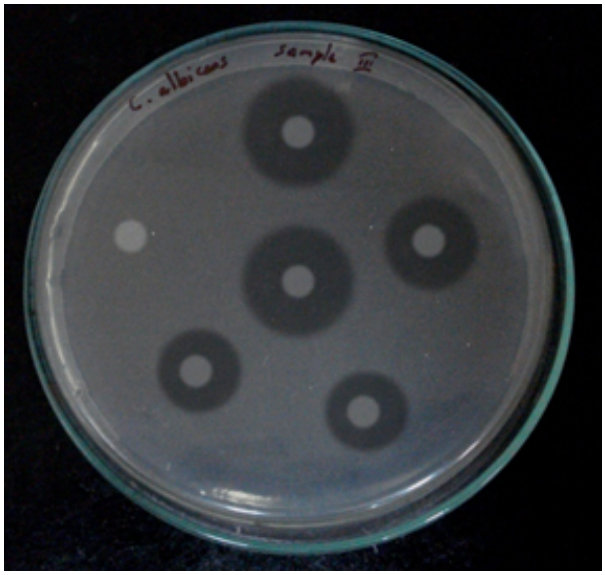

Figure 7: Anti-fungal activity of extract of A. indica against candida Albicans clockwise from top: 1000, 500, 250, 100 μg/disc and solvent (Dimethyl sulfoxide) control center: Amphotericin B (10 μg/disc) control

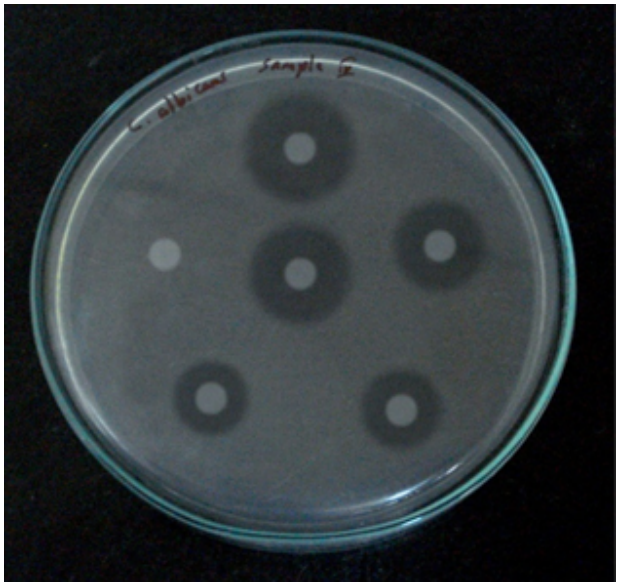

Figure 8: Anti-fungal activity of extract of V. vinifera against candida Albicans clockwise from top: 1000, 500, 250, 100 μg/disc and solvent (Dimethyl sulfoxide) control center: Amphotericin B (10 μg/disc) control

dentistry to give a better treatment effect. Here the extracts of a.arabica, t.chebula, a.indica, and v.vinifera are herbal medicine used in our study since large to moderate numbers of trees are found throughout the world, chiefly in light rainfall and deciduous forest areas. The contraindication of the extracts present in our studies includes systemic intake in pregnancy, weak digestion, dehydration, and heatstroke conditions (Chattopadhyay and Bhattacharyya, 2007). The contents present in our food extract are polyphenolic compounds, tannins, and anthraquinones. All the plant extracts in our study have been effectively used against inflammatory conditions of the oral cavity (Ncube *et al.*, 2008).

A variety of alternative products are promoted for oral and dental can be purchased over the counter. Herbal products in which the active ingredient has been extracted and have been studied for their effectiveness in toothpaste formulations, like polyphenols found in green tea which has proven antimicrobial effects.

The minimum inhibitory concentration of the plant extracts was determined using the dilution method as it has been reported in the studies to be more sensitive and is able to distinguish between anti-fungal and anti-bacterial effects and is used for quantitative determination.

In our present study MIC aqueous solution was determined and the test tube showed that the zone of inhibition was matched with the gold standard values of streptomycin when incubated in higher concentration, as the concentration became low the values gradually decreased and did not match the gold standard values of streptomycin and showed the anti-bacterial property for the streptococcus mutants. Whereas, in the antifungal property, the extracts of plants when incubated in high concentration matched the standard gold value of amphotericin B and higher values of extract a.indica and v.vinifera when compared to the amphotericin B value tested in candida Albicans organisms. As the available data suggest that the a.indica is used as an anti-microbial agent, but in our study, it proves its effectiveness as an anti-fungal agent, which adds more credit to our study.

The limitation of our study is they have taken concentration from 1000μg/ml and gradually decrease the concentration and has been tested, so further studies can be carried out with a higher concentration of more than 1000μg/ml to prove its anti-bacterial and anti-fungal effect of the plant extract we have used in our study.

CONCLUSION

As medicinal plant and herbal products are going advance hand in hand, in our study, we used four medicinal plants in which A.arabica and T.chebula were effective in anti-bacterial activity, and the extracts of A.indica and v.vinifera showed an effective anti-fungal activity. Further, studies can be carried out in higher concentrations.

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