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A Comparative Evaluation of Antimicrobial Efficacy of Neem, Aloe Vera and Turmeric on *Streptococcus Pyogenes*, *Streptococcus oralis* And *Staphylococcus Aureus* - An *In Vitro* Study

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Abstract

Background: Odontogenic infections are treated with an extended spectrum penicillin especially ampicillin. But, these pathogens acquires resistance due to wide spread usage, remains as a major challenge with the newer generation of antibiotics. So, natural products, from the ancient times, used to cure various ailments and emerges as alternatives to prevent the development of resistant species.

Aim: To evaluate the antimicrobial efficacy of neem, aloe vera and turmeric on *Streptococcus pyogenes(S.pyogenes)*, *Streptococcus oralis(S. oralis)* and *Staphylococcus aureus(S.aureus)*.

Methodology: Soxhlet extraction of *Azadirachta indica* (neem), *Curcuma longa* (turmeric) and direct extraction of *Aloe barbadensis* (aloe vera) aloe vera was done from the leaves. Petri plates containing 20 ml nutrient agar medium with 24 hr culture of *S. aureus*, *S. pyogenes* and *S. oralis* were subjected to concentrations of Neem, Turmeric and Aloe vera hydroalcoholic extracts (1000 µg/ml, 500 µg/ml, 200 µg/ml, and 100 µg/ml) and Ampicillin (positive control). These plates were incubated at 37°C for 24 hours. Antibacterial activity was assayed by measuring the diameter of the inhibition zone. The values were calculated using Graph Pad Prism 6.0 software.

Results: Ampicillin showed the maximum zone of inhibition on *S.pyogenes* and *S. oralis*, whereas *Neem* at 1000 ug/ml concentration showed a greater zone of inhibition against *S.pyogenes* and *S. aureus*, and *Aloe vera* showed maximum zone of inhibition on S. oralis.

Keywords: Cellulitis, Soxhlet extraction, Agar diffusion method

Introduction

Odontogenic infections are one of the most common infectious processes, often associated with host defense mechanism, bacterial virulence and regional anatomical structure involved [1]. The facultative aero-anaerobic like alpha-hemolytic streptococcus, Capnocytophaga, Staphylococci and Hemophilus influenza cause odontogenic infections [2]. Haemolytic Streptococcus (facultative anaerobe) cause cellulitis by producing hyaluronidase and streptokinase, thereby their inflammatory exudates spread in the subcutaneous and fascial planes resulting in gross swelling and sometimes leads to fatal situations due to sepsis [3].

Antimicrobial agents are synthetic chemical compounds that kill or inhibit the growth of other microorganisms. Ampicillin, an extended spectrum

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penicillin, found to be effective against gram positive and negative microorganisms. Its efficiency on S.viridians, *S.pyogenes* and **S**.aureus has been proved and hence it is commonly prescribed for odontogenic infections. But due to it's wide-spread clinical use, they acquire resistance to ampicillin [4]. Staphylococcus aureus (facultative anaerobe), hospital acquired infection, became resistant to both disinfectants and antibiotics [5]. Natural products, from the ancient times, not only cure various ailments but also prevent the development of resistance. Products like turmeric, propolis, ginger, honey, olive leaf extract, grapefruit seed extract, basil, and various oils such as thyme have proved to exhibit antimicrobial property.

Currently, phytochemistry are rapidly progressing and herbal products are becoming popular due to their antimicrobial activity and inhibition of resistance development [6]. The antimicrobial efficacy of each phytochemical of neem, turmeric and aloe vera on Staphylococcus aureus, Streptococcus pyogenes have been published in literature. But, the comparison of neem, aloe vera and turmeric at various concentrations has not been reported. The purpose of this study was to compare the antimicrobial efficacy of neem, Turmeric, and *A*loe vera on *S. Pyogenes, S.oralis* and *S.aureus.*

Methodology & Materials

The *in vitro* study was conducted in the Department of Pediatric and Preventive dentistry to compare the antimicrobial efficacy of phytochemicals Neem, Turmeric, and Aloe vera extracts on S. auerus, S. oralis, S. pyogenes using Agar well diffusion method. Phytochemical extraction included Petroleum ether, ethyl alcohol, Whatman No.1 filter paper, Soxhlet apparatus. Agar medium used were Nutrient broth and agar [Hi media labs, Mumbai].

The bacterial strains used were S. auerus - ATCC-902, S. oralis - ATCC-2696 and S. pyogenes - ATCC-1928 (IMTECH, Chandigarh).

Ampicillin was used as positive control against the natural alternatives.

Preparation of Hydroalcoholic Plant Extract

Collection of plant materials

The leaves of neem, turmeric, and Aloe vera were collected. They were washed thoroughly in running tap water to remove soil particles and other debris and shade dried for 15 days, grounded to a fine powder, stored in an airtight polythene container for further investigation.

Preparation of Neem and Turmeric extracts by Soxhlet extraction method

The crude powders (100 g) of turmeric and neem leaves were defated with 1 litre of petroleum ether (60°- 80°C) using Soxhlet apparatus. The extraction was carried out using 1000 mL of 100% ethyl alcohol (75°C) for 4 h. The samples were evaporated using rotary evaporator to remain with important ingredients in TRI-BIOTECH, Trichy.

Preparation of Aloe vera extract by direct method

Aloe vera (100 g) powder were immersed in hydroalcoholic solution overnight at 4°C and the extract was separated and filtered by Whatman No.1 paper and dried by rotary evaporator ingredients in TRI-BIOTECH, Trichy [7].

Agar - Well Diffusion Method

a. **Nutrient Agar Medium –** 2.8 g of the commercially available nutrient Agar Medium (HiMedia) dissolved in 100 ml of distilled water and autoclaved at 15 lbs pressure at 121°C for 15 minutes. Then they were mixed well and poured onto 100 mm petriplates (25-30ml/plate) while still molten.

b. **Nutrient broth –** 2.8 g of commercially available nutrient medium (HiMedia) dissolved in 100 ml distilled water and boiled till it dissolves completely. Then the medium were autoclaved at 15 lbs pressure (121ºC) for 15 minutes.

Petri plates containing 20 ml nutrient agar medium were seeded with 24 hr culture of bacterial strains of *S. aureus, S. pyogenes and S. oralis*. Wells of 6mm were cut and different concentration of neem, turmeric, and aloe vera, hydroalcoholic extracts (1000 µg/ml, 500 µg/ml, 200 µg/ml, and 100 µg/ml) were added. Ampicillin used as a positive control and hydro alcohol as the negative control. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells using vernier calliper. The values were tabulated and statistically analysed using one-way ANOVA with Graph Pad Prism 6.0 software (USA) [7].

Results

All statistical analysis were performed using Graph Pad Prism 6.0 software (USA) with power set at 80% before commencement of the study. One-way Anova test was used to compare the efficacy of phytochemicals at various concentrations. Probability value of $p < 0.05$ was considered statistically significant.

Table 1 showed the highest zone of inhibition in ampicillin over Neem against S. pyogenes and S. oralis which measures 40.0 ± 2.0 and 40.0 ± 4.0 respectively, whereas Neem at 1000 µg/ml concentration shows maximum effect on S.aureus of about 15.0 ± 1.5 with a high statistical significance of $p<0.05$. The lowest zone of inhibition was seen at 100ug/ml concentration. The effectiveness increased with increase in concentration of neem against all three microorganisms.

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Table 2 demonstrated highest zone of inhibition in ampicillin over Aloe vera which measures about 28.0 ± 6.0, 32.0 ± 4.0 and 10.0 ± 2.0 on S. pyogenes, S. oralis and S. aureus respectively with a higher level of significance at p<0.05. The lowest zone of inhibition was seen at 100ug/ml concentration. The effectiveness increased with increase in concentration of Aloevera against all three microorganisms.

Table: 3 revealed highest zone of inhibition in ampicillin over turmeric on S.pyogenes, S. oralis and S. aureus which measure about 34.0 ± 2.0, 30.0 ± 4.0 and 20.0 ± 3.0 respectively, with high statistical significance at p<0.05. The lowest zone of inhibition was seen at 100ug/ml concentration. The effectiveness increased with increase in concentration of turmeric against all three microorganisms.

Table 4 demonstrated maximum zone of inhibition in Neem on S. pyogenes at 1000ug/ml, 500ug/ml, 200ug/ml and 100ug/ml concentrations which measures about 30.0 ± 1.3, 24.0 ± 1.2, 20.0 ± 2.4 and 14.0 ± 1.15 respectively. The lowest zone of inhibition was observed in Aloe vera in all tested concentrations. The comparison between groups was found to be statistically significant.

Table 5 revealed maximum zone of inhibition about 32.0 ± 2.0, 24.0 ±0.25, 20.0 ± 1.5 and 12.0 ± 1.2 in Aloe vera on S. oralis at 1000ug/ml, 500ug/ml, 200ug/ml and 100ug/ml respectively with high level of statistical significance. The lowest zone of inhibition was observed in turmeric in all tested concentrations.

Table 1: Effect of Ampicillin and *Neem, hydroalcoholic extract on S. pyogenes, S. oralis* and *S. aureus at 100,200,500 and 1000ug/ml concentrations compared using one way ANOVA test.*

Name of the test sample	Zone of inhibition in mm		
	Mean ± SD		
	S. pyogenes	***S. oralis***	***S. aureus***
Ampicillin	**40.0 ± 2.0**	**40.0 ± 4.0**	11.0 ± 2.0
Neem (1000 µg/ml)	30.0 ± 1.3	32.0 ± 2.5	**15.0 ± 1.5**
Neem (500 µg/ml)	24.0 ± 1.2	26.0 ± 1.2	9.0 ± 2.0
Neem (200 µg/ml)	20.0 ± 2.4	20.0 ± 1	8.0 ± 1.0
Neem (100 µg/ml)	14.0 ± 1.15	14.0 ± 1.4	6.0 ± 1.5
	p<0.05	p<0.05	p<0.05

Table 2: Effect of Ampicillin and *Aloe vera hydroalcoholic extract on S.pyogenes , S. oralis* and *S. aureus at 100,200,500 and 1000ug/ml concentrations compared using one way ANOVA test.*

Name of the test sample	Zone of inhibition in mm		
	Mean ± SD		
	S. pyogenes	***S. oralis***	***S. aureus***
Ampicillin	**28.0 ± 6.0**	**32.0 ± 4.0**	**10.0 ± 2.0**
Aloe vera (1000 µg/ml)	16.0 ± 1.8	32.0 ± 2.0	9.0 ± 1.0
Aloe vera (500 µg/ml)	12.0 ± 1.75	24.0 ± 0.25	6.0 ± 1.0
Aloe vera (200 µg/ml)	10.0 ± 1.9	20.0 ± 1.5	5.0 ± 1.0
Aloe vera (100 µg/ml)	10.0 ± 3.4	12.0 ± 1.2	3.0 ± 2.0
	p<0.05	p<0.05	p<0.05

Table 3: Effect of *Ampicillin and turmeric hydroalcoholic extract on S. pyogenes , S. oralis* and *S. aureus at 100,200,500 and 1000ug/ml concentrations compared using one way ANOVA test.*

Name of the test sample	Zone of inhibition in mm		
	Mean ± SD		
	S. pyogenes	***S. oralis***	***S. aureus***
Ampicillin	**34.0 ± 2.0**	**30.0 ± 4.0**	**20.0 ± 3.0**
Turmeric (1000 µg/ml)	30.0 ±3.27	22.0 ± 0.5	14.0 ± 1.5
Turmeric (500 µg/ml)	24.0 ± 1.6	16.0 ± 3.5	12.0 ± 1.0
Turmeric (200 µg/ml)	12.0 ± 0.55	12.0 ± 1.5	10.0 ±1.5
Turmeric (100 µg/ml)	10.0 ± 2.0	10.0 ± 1.75	6.0 ± 1.0
	p<0.05	p<0.05	p<0.05

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Table 4: Effect of *Aloe vera, Neem* and *Turmeric hydroalcoholic extract on S. pyogenes at 100,200,500 and 1000ug/ml concentrations compared using one way ANOVA test.*

S. No	Name of the test sample	Zone of inhibition in mm for S. pyogenes			
		Mean ± SD			
		1000ug/ml	*500ug/ml*	*200ug/ml*	*100ug/ml*
1	Neem	**30.0 ± 1.3**	**24.0 ± 1.2**	**20.0 ± 2.4**	**14.0 ± 1.15**
2	Aloe vera	16.0 ± 1.8	12.0 ±1.75	10.0 ± 1.9	10.0 ± 3.4
3	Turmeric	29.0 ±3.27	23.0 ± 1.6	12.0 ±0.55	10.0 ± 2.0
		p<0.05	p<0.05	p<0.05	p<0.05

Table 5: Effect of *Aloe vera, Neem* and *Turmeric hydroalcoholic extract on S. oralis at 100,200,500 and 1000ug/ml concentrations compared using one way ANOVA test.*

S. No	Name of the test sample	Zone of inhibition in mm for S. oralis			
		Mean ± SD			
		1000ug/ml	*500ug/ml*	*200ug/ml*	*100ug/ml*
1	Neem	31.0 ± 2.5	22.0 ± 1.2	19.0 ± 1	11.0 ± 1.4
2	Aloe vera	**32.0 ± 2.0**	**24.0 ±0.25**	**20.0 ± 1.5**	**12.0 ± 1.2**
3	Turmeric	22.0 ± 0.5	16.0 ± 3.5	12.0 ± 1.5	10.0 ± 1.75
		p<0.05	p<0.05	p<0.05	p<0.05

Table 6: Effect of *Aloe vera, Neem* and *Turmeric hydroalcoholic extract on S. aureus at 100,200,500 and 1000ug/ml concentrations compared using one way ANOVA test.*

S. No	Name of the test sample	Zone of inhibition in mm for S. aureus			
		Mean ± SD			
		1000ug/ml	*500ug/ml*	*200ug/ml*	*100ug/ml*
1	Neem	**15.0 ± 1.5**	**12.0 ± 1.0**	**10.0 ±1.5**	**7.0 ± 1.5**
2	Aloe vera	9.0 ± 1.0	6.0 ± 1.0	5.0 ± 1.0	3.0 ± 2.0
3	Turmeric	14.0 ± 1.5	9.0 ± 2.0	8.0 ± 1.0	6.0 ± 1.0
		p<0.05	p<0.05	p<0.05	p<0.05

Table 6: showed highest efficacy in Neem at 1000ug/ml, 500ug/ml, 200ug/ml and 100ug/ml with a zone of inhibition measuring about 15.0 ± 1.5, 12.0 ± 1.0, 10.0 ±1.5 and 7.0 ± 1.5 respectively. The lowest zone of inhibition was observed in Aloe vera in all mentioned concentrations. The comparison between groups was found to be statistically significant.

Discussion

Cellulitis, an acute inflammatory condition of the skin, characterized by localized pain, erythema, swelling and heat and caused by indigenous flora of skin (eg.,S.aureus and S.pyogenes) or by a wide variety of exogenous bacteria. S. pyogenes, S. oralis and S. aureus are commonly associated with cellulitis. A punch biopsy study on cellulitis patients reported that there were beta-Hemolytic streptococci in 17 primary lesions, and coagulase-positive staphylococci in 13 patients, S. aureus in 50% of cases, and group A streptococci and/or S. aureus along with other gram-positive organisms seen in the remaining cases [8].

The treatment of cellulitis targeted against the causative organism.[9] The immediate start of antimicrobial therapy prevents bacteremia and spread of infection. Penicillin, amoxicillin, ampicillin, metronidazole and erythromycin were drug of choice [10]. Although pencillin is the first line of drug, its usage is reduced due to drug resistance and treatment failure [11]. Erythromycin drug action was found to be effective on odontogenic pathogens but its use limited due to resistance [12]. Metronidazole has bactericidal action against anaerobic species [13]. Ampicillin is similar to benzyl penicillin has its bactericidal action during the active multiplication stage, where it inhibits cell wall mucopeptide biosynthesis. Ampicillin has bactericidal activity action with all gram-positive, gram-negative aerobic and anaerobic bacteria. Therefore, was used as control in the present study. Because of its wide-spread use, these pathogens have acquired resistance to the drug [14].

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These synthetic agents were proven to cause various side effects [16] and also increase in morbidity and mortality due to treatment failures because of development of resistance [15]. Natural products could be an alternative, obtained from various sources like prokaryotic or eukaryotic microorganism, plants and animal organisms. Systematic screening can discover novel effective compounds of these natural products [17]. The study on the antimicrobial efficacy of each phytochemical - neem, turmeric and aloe vera at various concentrations on S. aureus and S. pyogenes have proved to be effective [18, 19, 20] but studies on S. oralis are sparse. In the current study, agar well diffusion method has been because they are simple, easy to reproduce, inexpensive, easy to read and interpret for evaluating the anti-microbial activity of plants or microbial extracts [21]. and the well-variant method is found to be more sensitive than the disc-variant [22].

In the present study, Ampicillin shows the maximum zone of inhibition on S. pyogenes and S. oralis and reports are similar with the study by Tadayoshi Ikebe et al [23] and Shiranee Sriskandan et al [24]. The study done by Melissa A. Foxley et al[25] and Patricia C. Harris et al[26] said that ampicillin was found to be effective on S.aureus and study by Serap Suzuk et al [27] also reported that Viridans group Streptococci (VGS) was found to be susceptible and were accordance with the current study Phytochemical extract of neem, turmeric and aloe vera were found to have antibacterial, antifungal and antiviral effect on wide variety of microbial species. Neem also has anti-inflammatory, antiseptic, astringent and analgesic properties [28]. Neem shows greater zone of inhibition at1000 ug/ml conc. than ampicillin against S. aureus. And efficacy seen even in low concentration of 100 ug/ml and the efficacy increases with the increase in concentration and were found to be similar to that reported by Bakkiyaraj S [29]. The neem revealed considerable efficacy against S. aureus and S. pyogenes in a study by Ajaba. M et al [30] and similarly in current study neem showed greatest zone of inhibition at various concentration. The study by Cai Y [31] used neem leaves on S. aureus and reported that neem was effective than aloe vera and also Vipul [32] in in vitro study also reported that neem exhibits antibacterial activity against both S. aureus and E. coli and both are similar to results of the current study Aloe vera has fleshy leaves filled with clear, viscous gel which shows maximum zone of inhibition against S. oralis. The studies by Agarry et al[33] and Stanley et al [19] had reported considerable zone of inhibition with the use of aloe vera against S.aureus which are found to be similar to the findings of the present study, but Abakar et al [34] reported no effect of aloe vera against S. aureus , which is contrary to the finding of this study.

In a study done by Safia et al [35] concluded that the ethanol leaf and root extracts of aloe vera gel has intended effect of antibacterial activity against both Gram-positive and as well as Gram-negative bacteria especially S.aureus and Saddiq [36] in SEM study found that the largest zone of inhibition zone against S. aureus with the use of aloe vera. Arab [37] reported that S. aureus showed significant zone of inhibition at 100 µl concentration of aloe vera extract. Pooja Agarwal [38] concluded that the plant extracts from aloe vera, Coriandrum sativum, Allium sativum and Zingiber officinale showed promising antimicrobial activity against harmful pathogenic strains and hence provide scope of developing more effective drugs. The current study results are similar to study by Haque [39] concluded that the extract of Aloe vera possesses antibacterial effect against the test pathogens as S.aureus, E.coli. Published data on antimicrobial efficacy against S. oralis has not been reported in literature. Turmeric showed a maximum zone of inhibition against S. pyogenes compared to neem and antibiotic and the results were similar to the study by Shabana and El-Adly [40].

In present study antibiotic ampicillin shows the maximum zone of inhibition on S. pyogenes and S. oralis, whereas neem showed greater antimicrobial efficacy against S. aureus at 1000 ug/ml concentration. In the comparison of various conc. of 100 ug/ml, 200 ug/ml, 500 ug/ml and 1000ug/ml of neem, aloe vera and turmeric, neem showed a greater zone of inhibition against S. pyogenes and S. aureus, and aloe vera showed maximum zone of inhibition on S. oralis. The present study reveals that phytochemicals from neem, aloe vera and turmeric exhibit considerable zone of inhibition which increases with increase in concentration of phytochemicals against S. aureus, S. pyogenes, S. oralis but less effective when compared to antibiotic ampicillin. So, further studies with in vivo trials are required to determine the efficacy of various concentrations of phytochemicals for confirmatory report.

Conclusion

The comparison of neem, aloe vera and turmeric in the present study reveals that neem and turmeric at a concentration of 100 ug/ml, 200 ug/ml, 500 ug/ml and 1000ug/ml, showed a greater zone of inhibition against S. pyogenes and S. aureus, whereas Aloe vera showed maximum zone of inhibition on S. oralis.

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