

ANTIMICROBIAL ACTIVITY OF GOTUKOLA LEAVES AND NEEM LEAVES - A COMPARATIVE INVITRO STUDY

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ABSTRACT:

Aim: The study was done to assess and compare the antimicrobial activity of gotukola leaves and neem leaves using different solvents against test pathogens. **Materials and method:** An experimental invitro study was conducted to assess antimicrobial activity of gotukola leaves and neem leaves. Test pathogens used in this study are *Streptococcus mutans*, *Lactobacillus acidophilus* and *Candida albicans*. Fresh gotu kola leaves and neem leaves were collected. The leaves were dried and powdered. 25gms of powdered neem leaves and gotu kola leaves were dissolved in 75ml of the solvents (i.e., Hexane, Ethyl acetate and Acetone) separately. The bottles were kept in the orbital shaking incubator at 37°C for about 3 days. On the 4th day, solvents were filtered and evaporate. The weighed extract was dissolved in 3 ml of dimethyl sulphoxide. Agar-well diffusion method was employed for microbiological analysis and the zone of inhibition of the growth was measured. ANOVA was used for statistical analysis. **Results:** Both neem leaves and gotukola leaves had an effect against *Streptococcus mutans* with neem leaves having more effect than gotukola leaves. Gotukola leaves had much better effect against *Lactobacillus acidophilus* than neem leaves with hexane as a solvent and showing no effect with ethyl acetate as a solvent. Gotukola leaves also showed mild effect against *Candida albicans* whereas neem leaves showed no effect. **Conclusion:** It can be concluded from the study that gotu kola leaves had a better effect than neem leaves against *Lactobacillus acidophilus* with hexane as a solvent and it was statistically significant ($P < 0.05$). Gotu kola leaves also have mild effect against *Candida albicans* with acetone, hexane and ethyl acetate as a solvent.

Key Words: Neem leaves, Gotu Kola leaves, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Candida albicans*.

INTRODUCTION:

Indian subcontinent is the treasure house of numerous plants and medicinal properties have been assigned to several thousands. Recently there is renewed interest in use of various Ayurvedic drugs for oral and dental health^[1]. According to the World Health Organization (WHO), about 65- 80% of the world's population in developing countries, due to the poverty and lack of access to modern medicine, depend essentially on plants for their primary healthcare. Historically, all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts, mixtures, etc. Even today, tribals and certain local communities in India practice herbal medicine to cure a variety of diseases and disorders^[2]. Herbal plants represent a rich source of antimicrobial agent^[3]. Medicinal use of plants range from the plant roots, bark, stem, flowers, leaves, seeds and extracts from the whole plants^[4].

Neem is an evergreen tree, cultivated in various parts of the Indian subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity^[5]. In dentistry, *Azadirachta indica* (neem) has been investigated, due to its antimicrobial potential against

oral micro-organisms especially those associated with gingivitis and periodontitis, and is concluded to be highly efficacious as an alternative to chlorhexidine in cases of periodontal disorders^[6]. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products^[7]. Neem (*Azadirachta indica*) tree has attracted worldwide prominence owing to its wide range of medicinal properties^[8].

Centella Asiatica (Gotu kola) is often called one of the miracle elixirs of life because legend has it that an ancient Chinese herbalist lived for more than 200 years as a result of using this herb^[9]. The plant has high

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medicinal value which makes it socially desirable. It is economically affordable and sustainable. In India it is commonly found in moist areas and in crop fields and other waste places upto an altitude of 600m. Thus, India is considered to be a rich emporium of drug plants, mainly used in preventive and curative medicine. Screening of medicinal plants for antimicrobial agents has gained much importance due to its reduced cost and lesser adverse reactions to plant preparations compared to modern conventional pharmaceuticals. Hence, there is a need to promote the traditional preventive measures that are acceptable, easily available, and cost effective [10]. Thus, the study was conducted to assess the antimicrobial efficacy of neem leaves and gotu kola leaves using different solvents against test pathogens.

MATERIALS AND METHODS:

Plant source

Gotu kola leaves and neem leaves were freshly collected. The leaves were washed with pure water and allowed to dry in sunlight for about 15 days and the dried leaves were powdered and sieved to get the fine grains. 25gms of powdered gotukola leaves and neem leaves were dissolved in 75ml of the solvents (i.e., Hexane, Ethyl acetate and Acetone) separately. The solution was poured separately in the amber coloured glass bottles and the bottles were labeled.

Crude solvent extract

The petri dishes were labelled and weighed individually and the weight was noted.

The filtered solution was poured in the weighed petriplates and kept in the autoclave for about 1 day. The petriplates were weighed again to get the exact amount of the extract weight. The weight of the extract was calculated by dividing the weight of the petriplates after adding the solution from the weight

The weight of the petriplates after adding the extract was also calculated.

The weight of the extract was calculated by subtracting the weight of petriplates before and after adding the extract.

Anti microbial assay

The leaves were tested against three pathogens which are present in the oral cavity under study.

Test organisms under study

They are *Streptococcus mutans*, *Lactobacillus acidophilus* and *Candida albicans*.

Agar-well diffusion test

Sterile Muller Hinton Agar plates for bacteria and Sabouraud Dextrose Agar for *Candida albicans* were

prepared and cultures were spreaded on the agar plates. Wells of about 6mm diameter were made in the agar surfaces. Leaf extract of neem and gotukola were poured into the wells of 5 agar surfaces of 6 plates (3 plates for neem leaves and 3 plates for gotu kola leaves) respectively. The positive control used for *Streptococcus mutans* and *Lactobacillus acidophilus* was erythromycin and fluconazole for the *Candida albicans*. The negative control used was dimethyl sulphoxide. The agar plates were incubated at 37°C for 24 hours. After incubation period, plates were removed and zones of inhibition were measured.

The diameter of the zone of inhibition of the growth were measured by the use of scale ruler in millimeter (mm) clear zones of inhibition indicated the susceptibility of the organism to the extracts while absence of such zones showed resistance or no inhibitory effect of extracts on the test organism (shown in Figures 1,2,3).

Determination of Minimum Inhibition Concentration value

The concentration of drug required to produce the effect is defined as the Minimum Inhibitory Concentration.

For determining minimum inhibitory concentration, the respective bacterial and Candidial strains from the stock was revived by plating on blood agar medium. After overnight incubation at 37°C, isolated colonies were selected and the identities of the organisms were confirmed. Isolated colonies were transferred to sterile Muller Hinton broth and Sabouraud dextrose broth for the bacterial and candidial strain respectively and once again incubated over night. An aqueous solution of 15% concentration was prepared from the neem powder as the stock solution. 100 ul. of the MHA broth was added in each of six MIC wells per strain. For Candida strain 100 ul of the sabouraud dextrose broth was added in each of six MIC wells. In the first MIC wells containing 100 ul broth, 100 ul of the stock was added. After mixing well 100 ul was transferred to the second MIC well. This was continued till the last (6th) well. From the last tube 100 ul final solution was discarded. By following this serial dilution, the concentration of the neem powder was achieved as the following 100mg, 50mg, 25mg, 12.5mg, 6.25mg and 3.1mg respectively. Following the 18 to 24 hours of incubated exposure, the tubes containing the challenge microorganism/product/medium suspension were examined to determine the highest dilution of product (and conversely, the lowest concentration of product) that completely inhibits growth of the microorganism, as determined by the naked eye. This dilution value (and/or product concentration value) was recorded as the Minimum Inhibitory Concentration value.

Statistical Analysis: ANOVA was used for the statistical analysis.

RESULTS:

The zone of inhibition of gotu kola leaves and neem leaves in millimeter is shown in tables 1, 2.

Table 1: Antimicrobial efficacy of the crude solvent extracts of gotu kola leaves against the test pathogens

MICRO-ORGANISMS	SOLVENTS (Zone of inhibition in mm)			P VALUE
	Acetone	Ethyl Acetate	Hexane	
<i>Lactobacillus acidophilus</i>	12	NA	14	0.000***
<i>Streptococcus mutans</i>	8	8	10	0.001***
<i>Candida albicans</i>	10	10	10	1.000 ⁺
NA - No Activity, P<0.001 -Statistically very highly significant				

Table 2: Antimicrobial efficacy of the crude solvent extracts of neem leaves against the test pathogens

MICRO-ORGANISMS	SOLVENTS (Zone of inhibition in mm)			P VALUE
	Acetone	Ethyl Acetate	Hexane	
<i>Lactobacillus acidophilus</i>	15	14	12	0.000***
<i>Streptococcus mutans</i>	16	14	12	0.000***
<i>Candida albicans</i>	NA	NA	NA	NA
NA - No Activity.				

Minimum inhibitory concentration

The MIC value for the neem leaves extract with ethyl acetate, acetone and hexane was determined as 100mg/ml, 50mg/ml and 25mg/ml respectively and showed resistance with other concentrations against *Streptococcus mutans*.

The MIC value for the gotu kola leaves extract with hexane was determined as 50mg/ml and showed resistance with ethyl acetate and acetone solvents at all concentrations against *Streptococcus mutans*.

The MIC value for the neem leaves extract with ethyl acetate, acetone and hexane was determined as 100mg/ml, 100mg/ml and 25mg/ml respectively and showed resistance with other concentrations against *Lactobacillus acidophilus*.

The MIC value for the gotu kola leaves extract with acetone and hexane was determined as 12.5mg/ml

each respectively and showed resistance with ethyl acetate solvent against *Lactobacillus acidophilus*.

The MIC value for the neem leaves extract with acetone, ethyl acetate and hexane showed resistance with all the concentrations against *Candida albicans*.

The MIC value for the gotu kola leaves extract with ethyl acetate and hexane was determined as 12.5mg/ml and showed resistance with acetone solvent against *Candida albicans*.

Figure 1: Culture plates of *Lactobacillus acidophilus*

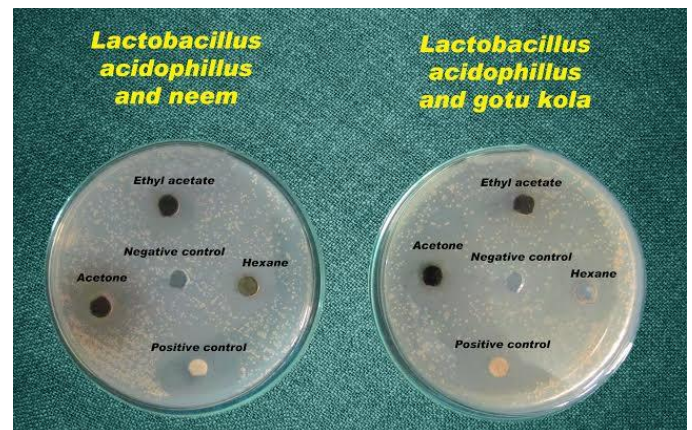


Figure 2: Culture plates of *Streptococcus mutans*

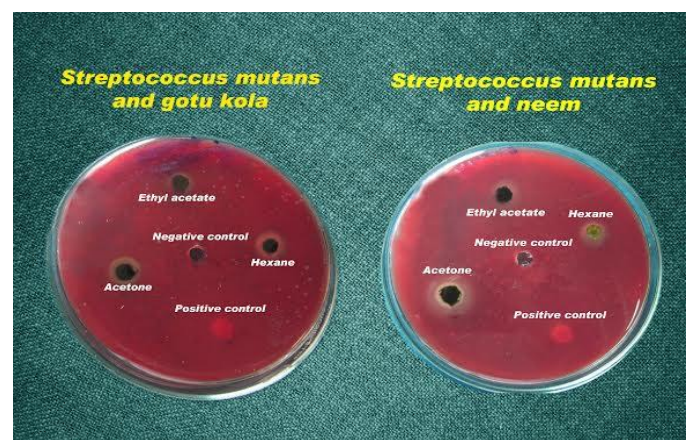
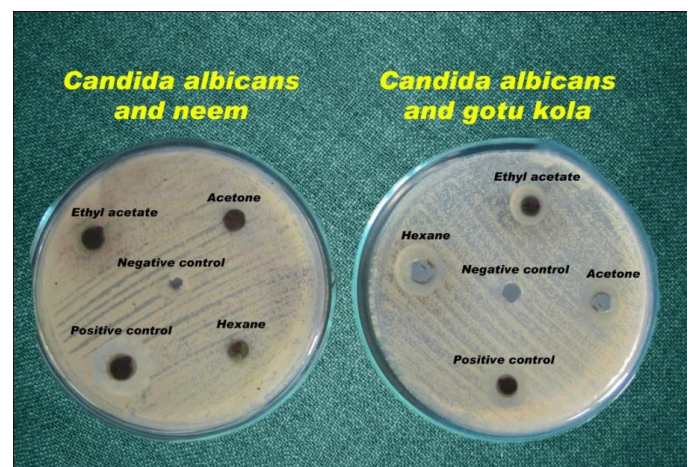


Figure 3: Culture plates of *Candida albicans*



DISCUSSION:

In the present study neem leaves showed no zone of inhibition against *Candida albicans* with acetone, ethyl acetate and hexane as solvents respectively but in a study conducted by Aarti bohora et al neem leaves extract showed an inhibition zone of 7.1mm against *Candida albicans* when absolute ethanol was used as a solvent.^[6] Methodology of present study followed the standard established agar well diffusion method.

Candida albicans showed resistance to neem leaves with all the solvents in the present study whereas in a study conducted by Mahmoud et al (2011)^[8] neem leaves showed an inhibition at 9.84% concentration against *Candida albicans* and in a study conducted by Nayak Aartati et al (2011)^[12], it was shown that the Minimum Inhibitory Concentration (MIC) of aqueous neem extract and alcoholic neem extract against *Candida albicans* was 7.5% and 3.75% respectively. The differences noted may be due to the difference in solvents used. Several pharmacological activities and medicinal applications of various parts of neem are well known.

Bitter taste associated with this plant can be altered by different formulations due to addition of sweeteners and flavours to increase the patient compliance and acceptability.

The result obtained in this in vitro study showed that neem leaf extract with acetone, ethyl acetate and hexane as solvents is not a viable medicament against *Candida albicans*.

The finding of the present study suggests the presence of no growth is an indication of high effectiveness of the extract whereas presence of growth indicates the less effectiveness of the extract.

In the present study, neem leaves showed an inhibition zone of 16mm, 14mm and 12mm against *Streptococcus mutans* with acetone, ethyl acetate and hexane as solvents respectively whereas in a study conducted by Widowati Siswomihardjo et al (2007)^[14], neem leaves showed a mean inhibition zone of 3.8mm at 20% concentration when ethanol was used as a solvent. Neem leaves had the active component of azadirachtin which is responsible for the anti-bacterial properties.

Streptococcus mutans showed sensitivity to neem extract with ethyl acetate at concentration of 100mg/ml (10%) with acetone at concentration of 50mg/ml (5%) and with hexane at the concentration of 25 mg/ml (2.5%). In a study conducted by Prashant et al (2014)^[11] neem stick extract was effective at 50% concentration on *Streptococcus mutans*.

Lakshmi.T et al (2012)^[15] conducted the study to evaluate the antibacterial activity of ethanolic leaf

extract of neem against selected acidogenic oral bacteria and proved that the ethanolic neem leaf extract does not show antibacterial activity against *Lactobacillus acidophilus* whereas in the present study, the MIC value for the neem leaves extract with ethyl acetate, acetone and hexane was determined as 100mg/ml(10%), 100mg/ml(10%) and 25mg/ml (2.5%) respectively and showed resistance with other concentrations.

The differences noted may be due to the differences in the solvents used.

In the present study, gotu kola leaves showed an inhibition zone of 8mm, 8mm and 10mm against *Streptococcus mutans* with acetone, ethyl acetate and hexane as solvents respectively and in a study conducted by Dora Imefon Udoh et al (2012)^[4] inhibition zone of 12mm, 12.5mm, 14.9mm, 15.4 and 16.4mm was obtained at 100(10%), 200(20%), 300(30%), 400 (40%) and 500mg/ml (50%) of concentration against *Streptococcus pyogenes* when ethanol was used as a solvent. In a study conducted by Varahalarao vadlapudi (2012)^[13], Gotu kola leaves extract showed no zone of inhibition against *Streptococcus mutans* when methanol and chloroform were used as a solvent.

The limitation of study is that it is a preliminary study, further fractionation and purification assays have to be carried out to identify the bioactive compounds present in neem and gotu kola leaves which are responsible for the antibacterial and antifungal activity. Randomized controlled clinical trials are also needed to evaluate the long term success of these products.

CONCLUSION:

It has been concluded from the study that gotu kola leaves seem to have a better effect than neem leaves against *Lactobacillus acidophilus* with hexane as a solvent.

Gotu kola leaves seem to have a less effect than neem leaves against *Streptococcus mutans* and have a promising future with effect against *Candida albicans* compared to neem leaves

Since herbal medicines possess anti microbial, anti inflammatory activity, it can be effectively used as a prophylactic and therapeutic agent against acidogenic oral bacteria. Compared to other therapeutic agents, herbs like neem and gotu kola have lesser side effects and are easily available and economical.

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