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Original Research Article

Phytochemical screening of *Acacia nilotica* extract: an in vitro study

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ABSTRACT

Background: The present study aim was to identify the presence of phytochemical screening of flower extract of *Acacia nilotica*.
Methods: The flower extract of *Acacia nilotica* was prepared using Soxhlet apparatus. The preliminary phytochemical analysis of methanol flower of *Acacia nilotica* was carried out by using simple chemical tests and compared with ethanol and aquesous extract of the same plant. The antimicrobial testing was done using agar well diffusion method. Minimum inhibitory concentration (MIC) was carried out by micro dilution assay and minimum bactericidal concentration (MBC) was done using colony forming unit (CFU) method. SPSS version 20 was used for statistical analysis.
Results: The results of phytochemical screening of methanol flower extract of *Acacia nilotica* showed the presence of alkaloid, flavonoid, glycosides, tannin, terpenoids, steroids whereas ethanol shows the presence of flavonoid, glycosides, tannin, terpenoids, saponin, steroids and aqueous extract shows presence of alkaloid, flavonoid, glycosides, tannin, terpenoids, saponin, steroids. The antibacterial activity of the (flower) extract of *Acacia nilotica* showed relatively high zone of inhibition (21 mm,19 mm) at 40 mg/ml against *S. mutans* and *L. acidophillus*. The MIC OD at 600 nm showed maximum inhibition at 40 mg/ml against *S. mutans* and *L. acidophillius* was about 78% and 91% respectively.
Conclusions: The present study signifies the phytochemical screening of *Acacia nilotica* which could be considered in the evolution of an indigenous herbal mouth rinse or toothpaste as the formulation inhibited all the micro-organisms tested in this study at low concentrations.

Keywords: *Acacia nilotica*, Dental caries, Phytochemicals

INTRODUCTION

Acacia nilotica tree has multipurpose benefits known as the most exclusive source of life saving and herbal drugs worldwide.[1] This plant is significant due to presence of useful natural organic groups like alkaloids, flavonoids, tannins, phenols, tannis, saponins, proteins, carbohydrates and amino acids.[2] This legume tree occurs in Asia, Australia, Africa and many parts of the world commonly. Almost all parts of *Acacia nilotica* like bark, root, gum, leaves and flowers have been used for diabetes, eczema, cough, astringent, dysentery and multipurpose diseases.[1]

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contribution to human health and wellbeing.[3] Medicinal plants have been identified and used throughout human history.[4] Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators.[5]

Hence, this *Acacia nilotica* is used for treatment of various diseases.[6] It serves as the source of polyphenols. The plant contains a profile of a variety of bioactive

components.[7] A number of medicinal properties have acute diarrhoea.[8] The bark of plant is used extensively for colds, bronchitis, diarrhea, bleeding piles and leukoderma.[9] Pods and tender leaves are given to treat diarrhea and are also considered in folk medicine to treat diabetes mellitus. The present study was conducted to screen the different phytochemicals present in the methanol extract and compared with ethanol and aqueous extract of flower of *Acacia nilotica*.

METHODS

Study design and study setting

This in vitro experimental study was conducted from February to July 2022 at the research lab, Dextrose technologies Pvt. Ltd., Bangalore.

Plant material

The flower was collected from the local areas which was identified and authenticated by the department of dravyaguna, SDM Institute of Ayurveda and hospital, Bangalore.

The flower was washed thoroughly with distilled water at room temperature, and dried at Hot air oven at 24 to 72ºC. The dried flower uniformly ground using an electric grinder.

Preparation of extract

10 gm of powdered sample was filled into a thimble and subjected to Soxhlet extraction using 150 ml methanol as solvent.[10] The flower extract was concentrated using rotary evaporator and used for further analysis.

Antimicrobial testing

The antimicrobial efficacy of plant extract was determined by using (Agar well diffusion) diffusion method.[11] Wells of 6 mm diameter were punched on specific agar media. About 100µl of pre-cultured test organisms-*Streptococcus mutans* and *Lactobacillus acidophillus*, were spread onto the agar plates. Various concentrations (5 mg, 10 mg, 15 mg, 20 mg, 30 mg, 40 mg) of samples were loaded into the wells. Duplicated Bacterial plates were incubated at 37ºC for 24 hours, and the inhibition zones were measured and tabulated.

Minimum inhibitory concentration (MIC)

The MIC of herbal extract against *S. mutans* and *L. acidophillus* were determined by micro dilution assay. A separate microplate was used for each bacterial species assessed and were incubated for 24 hours at 37ºC. After incubation, the optical density of each well was evaluated using a spectrophotometer (Labman, India) at 600 nm after the plate incubation at 37ºC for 24 hours. MIC of the extract that repressed the turbidity was determined.

Minimum bactericidal concentration

The MBC was determined by sub culturing of the wells that was displayed no perceivable growth on a sterile agar plate. 100 µL of the bacterial solutions that was considered as the MIC and higher concentrations were grown on tryptone soya agar plates and the plates were incubated for 24 hours at 37ºC. Each plate was examined for growth at the conclusion of incubation period both by the naked eye, by Colony forming units (CFUs) which was calculated on a grid.[12] The MBC value was concluded as the lowest concentration which showed no apparent growth on agar plate. Data entry and statistical analysis SPSS (statistical package for social sciences) version 20 [IBM SPASS statistics (IBM corp. released 2011)] was used to perform the statistical analysis.

RESULTS

The results of phytochemical analysis of flower extract of *A. nilotica* has shown in Table 1 which reveals the presence of certain secondary metabolites such as alkaloids, flavonoid, glycosides, tannins, terpenoids and steroids while Saponin was absent in it, when compared with ethanol and aqueous extract ,ethanol extract showed the presence of flavonoid, glycosides, tannins, terpenoids, steroids, saponin while alkaloid was absent in it, and aqueous extract showed the presence of alkaloid, flavonoid, glycosides, tannins, terpenoids, steroids, saponin.

Table 1: Phytochemical analysis of flower extract of *Acacia nilotica*.

Phytochemicals	Methanol extract	Ethanol extract	Aqueous extract
Alkaloid Iodine test	++++	–	+
Flavonoid Alkaline reagent test	++++	++	++
Glycosides Keller-kiliani test	+++	+++	++
Tannin Ferric chloride test	++	+++	+++
Terpenoids Salkowski test	+	++	++
Saponin Froth test	–	+++	+++
Steroids Salkowski test	++	+	+

The inhibition zone flower extract of *A. nilotica* was illustrated in Table 2. The zones of inhibition at 5 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml was sized up 6 mm, 8 mm, 10 mm, 12 mm, 15 mm and 21 mm respectively.

Table 2: Zone of inhibition of *Acacia nilotica* against *S. mutans* and *L. acidophillus.*

Test organisms	Zone of inhibition (mm) concentration						
	5 mg/ml	5 mg/ml	10 mg/ml	10 mg/ml	20 mg/ml	20 mg/ml	40 mg/ml
S. mutans	7	6	9	8	11	12	19
L. acidophillus	6	8	10	9	12	13	20

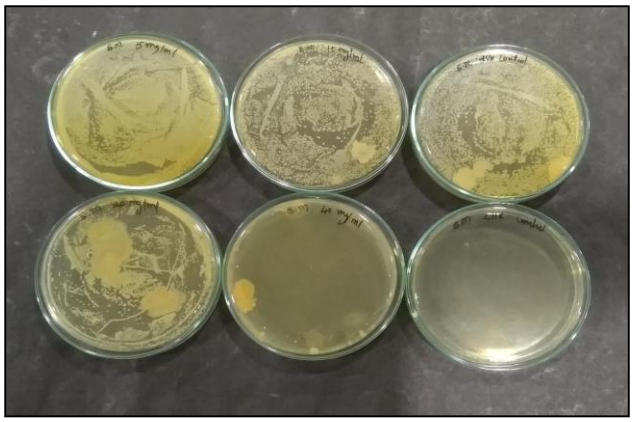

Figure 1: MIC and MBC of *S. mutans.*

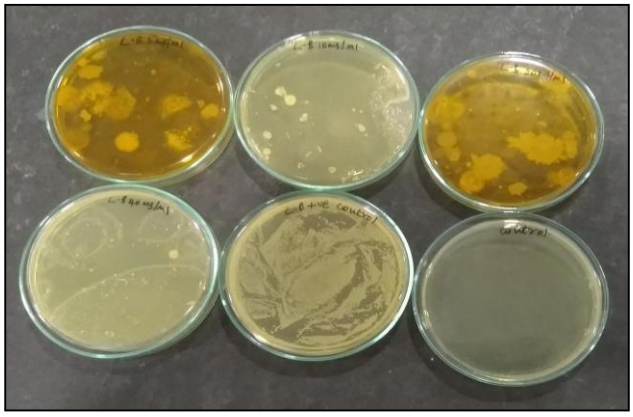

Figure 2: MIC and MBC of *Lactobacillus acidophilus*.

The results revealed that the MIC OD at 600 nm showed maximum inhibition at 40mg/ml against *S. mutans* and *L. acidophillius* was about 78% and 91% respectively. MBC exhibited against the *S. mutans* and *L. acidophillus* of flower extract at 40 mg/ml produced 1 and 2 CFU respectively (Figure 1 and 2).

DISCUSSION

Phytochemical screening of methanol, ethanol and aqueous extract of *A. nilotica* flower from Table 1 indicate the presence of certain secondary metabolites such as alkaloids, glycosides, cardiac glycoside, steroids, saponins, tannins, and terpenoids which may be responsible for the treatment of various disease in human body. The preliminary phytochemical screening investigation of *A. nilotica* may help in the recognition of bioactive compounds and it may lead to the discovery and development of new drugs. These tests will also facilitate separation of pharmacologically active chemical compounds. Therefore *A. nilotica* flower contain many secondary metabolites which are responsible for various medicinal properties and will be of great importance in phytomedicine like alkaloids which are reported to have many pharmacological activities such as analgesic, antimicrobial, anti-inflammatory anti-malarial activity. Tannins may be responsible for the antioxidant activities or free radical scavenging activities and heart diseases prevention as reported by Mamta et al.[3] Steroids are very important compounds due to their relationship with compounds such as sex hormone. The presence of steroids is suggestive of anti-inflammatory activity and blood cholesterol reducing capacity.

Deshpande and Kadam revealed that the preliminary phytochemical screening of ethanol and petroleum ether extract of stem bark of *Acacia nilotica* showed the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, cardiac glycosides and anthraquinone in both ethanol and ether extracts while fixed oils and fats, proteins and amino acids were absent.[4] In our study ,the phytochemical analysis done on methanol extract and compared with ethanol and aqueous extract of flower *Acacia nilotica*, results revealed aqueous extract showed the presence of all phytochemical tested whereas methanol and ethanol extract showed the absence of saponin and alkaloid respectively.[13]

Ghanghro et al also confirmed presence of alkaloids, flavonoids, tannins, phenols, tannins, saponins and amino acids and they also found heavy metals like nickel (Ni), chromium (Cr), zinc (Zn), cobalt (Co), cadmium (Cd), copper (Cu), manganese (Mn) and iron (Fe).[1] The most perilous metal like Fe (3.97 ppm), Cd (below detection limit) and Mn (0.06 ppm) were detected within the permissible limit in acacia flower. This present study showed presence of all phytochemicals with aqueous extract of flower *Acacia nilotica*.

Arshad et al revealed that the methanol extract of *A. nilotica* showed significantly highest zone of inhibition (18.00±1.00 mm, 20.00±1.15 mm and 16.67±0.67 mm) and MIC (0.3125, 0.3125 and 0.15625 mg/ml) followed by acetone and aqueous extracts against *S. mutans*, *S. mitis* and *P. intermedia* respectively.[8] The current study found that the flower extract of *A. nilotica* showed significantly higher antimicrobial efficacy in terms of zone of inhibition and MIC when compared with bark extract against *S. mutans* and *L. acidophillus*.

Abdulhamid et al Phytochemical constituents present in the crude leaves extract of *A. nilotica* are flavonoid, tannins, saponins, glycosides, alkaloids, cardiac glycosides, steroids, anthraquinones, terpenoids was present and balsams was absent in it.[14] In the present study flower extract showed the presence of secondary catabolites such as alkaloid, flavonoid, glycosides, tannin, terpenoids, steroids, saponin was absent in it.

Attahiru et al showed the preliminary qualitative phytochemicals screening of crude methanol extracts of *A. nilotica* leaves showed positive test for alkaloids, glycosides, cardiac glycoside, steroids, saponins, tannins, anthraquinones, flavonoids, terpenoids and phenol.[15] Whereas in this study phytochemicals screening of crude methanol extracts of *A. nilotica* flowers showed positive test for flavonoid, tannins, glycosides, alkaloids, cardiac glycosides, steroids.

The present study used crude extracts of the plants rather than purified compounds on *S. mutans* and *L. acidophilus*. The experiment was done in duplicate sets using the plant extracts obtained in one particular season in a year. The phytochemical constituents vary with seasons. The quantitative assay of each of these extracts will highlight the bioactive compound present in high concentrations. In this background, the results of this study are only preliminary and further in vitro studies using plant extracts in different seasons, purified compounds of these plants is the need of the hour. The evaluation of these extracts on secondary and tertiary plaque colonizers would enable us to evolve a new strategy that can simultaneously inhibit both dental caries and plaque microorganisms. The efficacy of these extracts in the form of mouth rinse under in vivo conditions is required to validate the results of this study.

CONCLUSION

The findings of this study suggest that the flower extracts of *A. nilotica* showed the presence of secondary metabolites such as alkaloids, flavonoid, glycosides, tannin, terpenoids, steroids. Thus, these plants could serve as potential sources of antimicrobial and antifungal agents which might be possibly due to presence of phytochemical constituents in the plant extracts, therefore helps as prophylactic and therapeutic agent for oral diseases.

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