

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *ACACIA NILOTICA* AGAINST *STREPTOCOCCUS MUTANS*

*DESHPANDE S.N., KADAM D.G.

Department of Microbiology, D.B.F. Dayanand College of Arts and Science, Solapur, Maharashtra. Email: seemadeshpande6@gmail.com.

Received: 23 Oct 2012, Revised and Accepted: 01 Dec 2012

ABSTRACT

Acacia nilotica commonly known in India as Babul has wide range of medicinal uses. In the present study, preliminary phytochemical screening of ethanol and petroleum ether extract of stem bark of *Acacia nilotica* was carried out. It revealed the presence of alkaloids, carbohydrates, saponins, Tannins, Flavonoids, cardiacglycosides and anthraquinone in both ethanol and ether extracts while fixed oils and fats, proteins and amino acids were absent. The antimicrobial activity of the extracts was determined by agar diffusion method. The ethanol extract showed more significant activity against *Streptococcus mutans* as compared to petroleum ether extract. The minimum inhibitory concentration of ethanol extract was 5 mg/ml while 10 mg/ml for ether extract. The results indicate that *Acacia nilotica* could be used as a source of antimicrobial agents to treat dental caries.

Keywords: Phytochemical screening, Agar diffusion, Antimicrobial activity, *Streptococcus mutans* *Acacia nilotica*.

INTRODUCTION

Dental caries is a localized infectious disease that leads to loss of teeth. Individuals heavily colonized by cariogenic bacteria are considered to be at high risk for dental caries[5]. *Streptococcus mutans* is considered to be the main cause of dental caries. It is an acidogenic bacterium that colonize the oral cavity[8]. Several antibacterial agents such as Chlorhexidine, Fluorides and various antibiotics are commercially available that can be used to prevent dental caries. However, these have been reported to have undesirable side effects like nausea, vomiting, tooth staining[3, 11]. Hence there is a constant search for alternative products for management of dental caries. Natural phytochemicals isolated from plants used in traditional medicine are good alternatives to synthetic chemicals[12]. About 80% of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants[7].

Acacia nilotica [Family-Mimosaceae] is a medium size thorny tree found in the drier parts of India. It has yellow mimosa like flowers and long grey pods constricted between seeds. The bark and branches are dark with fissures. The branches bear spikes about 2 cm long. The leaves are fine and densely hairy with 3-6 pairs of pinnate consisting of 10-20 pairs of leaflets that are narrow with parallel margins and are rounded at the apex with a central midrib closely crowded[9]. The powdered bark of the plant with little salt is used for treating acute diarrhea[6]. The bark is also used extensively for colds, bronchitis, diarrhea, bleeding piles and leucoderma[4]. The tender twigs are used as tooth brushes[10]. These literature reports have indicated therapeutic potential associated with *Acacia nilotica* plant constituents. The present investigation was undertaken for phytochemical analysis and evaluation of *Acacia nilotica* against *Streptococcus mutans*, the causative agent of dental caries.

MATERIAL AND METHODS

Plant material

The bark of the plant *Acacia nilotica* was collected from Smruti van Solapur, Maharashtra, India. It was identified and authenticated in the P.G. Department of Botany D.B.F. Dayanand college of Arts and Science, Solapur.

Test Microorganism

Pure culture of *Streptococcus mutans* was obtained from MTCC, Chandigarh, India.

Preparation of plant extract

The stem bark of *Acacia nilotica* was washed under running tap water. It was then dried under shade and ground into coarse powder

in the electronic grinder. Fifteen grams of powder was then extracted in ethanol [150 ml] and petroleum ether [150ml] by using Soxhlet method. Twelve cycles were done. The solvent was removed by evaporation at room temperature [28±2°C]. The extracts were kept in freeze until further use.

Phytochemical Analysis of Extract [13]

Detection of Alkaloids

Solvent free extract [50 mg] was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various reagents as follows.

1. **Mayer's test** -To a few milliliter of filtrate, a drop of Mayer's reagent is added by the side of the test tube. A white or creamy precipitate indicates the test as positive.
2. **Wagner's test** -To a few milliliter of filtrate, few drops Wagner's reagent are added by the side of the test tube. A reddish brown precipitate confirms the test as positive.
3. **Hager's test**—To a few milliliter of filtrate 1 or 2 ml of Hager's reagent is added. A prominent yellow precipitate indicates the test as positive

Detection of Carbohydrates

Benedict's test - To a 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 minutes. A characteristic colored filtrate indicates the presence of sugar.

Detection of Amino acids and proteins

The extract [100mg] was dissolved in 10 ml distilled water and filtered through Whatman no.1 filter paper and the filtrate was subjected to test for proteins and amino acids.

Biuret test - Two ml of filtrate was treated with one drop of 2% copper sulphate solution. To this 1ml. of ethanol was added followed by excess of potassium hydroxide pellets. Pink color in the ethanol layer indicates presence of proteins.

Ninhydrin test - Two drops of ninhydrin solution were added to 2 ml. of aqueous filtrate. A characteristic purple color indicates the presence of amino acids.

Detection of Saponins

Foam test -The extract [50mg] was dissolved in 20 ml. of distilled water. The suspension was shaken in a graduated cylinder for 15 minutes. A two cm. layer of foam indicates the presence of Saponins.

Detection of Tannins

Ferric chloride test

The extract [50mg] was dissolved in 5 ml of distilled water. To this few drops of 5% Ferric chloride were added. A dark green color indicates the presence of tannins.

Detection of flavonoids

Magnesium and hydrochloric acid reduction test

The extract [50 mg] was dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid [drop wise] were added. If any pink to Crimson color develops presence of flavonoids was inferred.

Detection of anthraquinones

The extract [50mg] was dissolved in distilled water. To 2 ml of extract, 1ml dilute ammonia solution was added and shaken vigorously. Pink color in ammonia layer indicates presence of anthraquinones.

Detection of Cardiac glycosides

Killer kiliani test

The extract [50mg] was dissolved in distilled water and then filtered. To 2 ml of filtrate 1ml of glacial acetic acid and a drop of Ferric chloride and a drop of concentrated sulfuric acid was added. Green blue color to upper layer and reddish brown color at the junction of two layers indicates the presence of cardiac glycosides.

Detection of fixed oils and fats

Spot test-A small quantity of extract was pressed between two filter papers. Oilstain on the paper indicates the presence of fixed oils.

Antibacterial activity of Plant Extract

Antibacterial activity of Plant Extract was determined by agar cup method. For this fresh [overnight] isolated colony of *Streptococcus mutans* was suspended in sterile saline to get turbidity of 0.5 McFarland standard. 0.1 ml. of this suspension was spread aseptically on sterile Muller Hinton agar medium. Then the wells [8 mm diameter] were bored by sterile cork borer. 0.2 ml. of each extract [100 mg /ml in 10% DMSO] was added to the wells. It was allowed to diffuse in freeze for 20 minutes. 10 % DMSO in one of the wells was used as negative control. Standard antibiotic gentamycin disc [10 microgram, Hi media] was also tested for comparative efficacy. After diffusion of extract the plates were incubated at 37 °c for 24 hours. Zones of inhibition were then measured in mm. For each extract, three replicates were maintained.

Determination of minimum inhibitory concentration [MIC]

Tube dilution method was done to determine minimum inhibitory concentration of the extracts. A series of two fold dilutions of each extracts ranging from 10 mg /ml to 0.3 mg/ml were made in Muller Hinton broth. 0.1 ml of *Streptococcus mutans* suspension matched to 0.5 McFarland standard was seeded into each dilution. Two controls were maintained for each test batch. These included tube containing extract and growth medium without inoculum and organism control. i.e. tube containing the growth medium and inoculums. The tubes were incubated at 37°C for 24 hours and checked for turbidity. Minimum inhibitory concentration was determined as the highest dilution of the extract that showed no visible growth.

RESULTS

Physicochemical characteristics of extracts:

Physical characteristics of ethanol and petroleum ether extracts of stem bark of *Acacia nilotica* have been depicted in table 1. Ethanol extract consisted of brown colour, dry crystalline in consistency and agreeable odor while petroleum ether extract was dark brown colored with dry crystalline consistency and agreeable odor.

Table 1: Physical characteristic of extracts of stem bark of *Acacia nilotica*

Solvent Used	Physical characteristics		
	Color	odor	consistency
Ethanol	Brown	Agreeable	Dry crystalline
Petroleum ether	Dark brown	Agreeable	Dry crystalline

The percentage yield [table 2] of ethanol extract was 8.3% and that of petroleum ether was 4.6%.

Table 2: Percentage yield of extracts of stem bark of *Acacia nilotica*

Solvent used	Weight of dry powder [G]	Weight of dry extracts [G]	Percentage yield
Ethanol	15	1.25	8.3
Petroleum ether	15	0.69	4.6

Phytochemical analysis

Table 3 shows the results of phytochemical screening of ethanol and petroleum ether extract of stem bark of *Acacia nilotica*. Both the extracts contained alkaloids, carbohydrates, saponins, tannins, flavonoids, anthraquinones and cardiac glycosides while proteins, amino acids, fixed fats and oils were absent in it.

Table 3: Phytochemical analysis of extracts of stem bark of *Acacia nilotica*

Phytochemical	Ethanol extract	Petroleum ether extract
Alkaloids		
Meyers test	--	--
Wagner test	+	+
Hangers test	-	-
carbohydrates	+	+
saponins	+	+
proteins	-	-
Amino acids	--	--
antraquinones	+	+
Tannins	+	+
Flavonoids	+	+
Fixed oils & fats	--	--
Cardiac glycosides	+	+

Antibacterial activity

The results of antibacterial activity of extracts are shown in table 4. The ethanol extract exhibited high degree of activity than petroleum ether against *Streptococcus mutans*. This might be due to less amount of active compound extracted in petroleum ether extract. The zone of inhibition of gentamycin against *Streptococcus mutans* was 34mm.

Table 4: Antibacterial activity of extracts of stem bark of *Acacia nilotica* against *Streptococcus mutans*.

Solvent Used	Zone of inhibition [MM.]± SD
Ethanol	31±0.7
Petroleum ether	17.5±0.8

Table 5: Minimum inhibitory concentration of the extracts of stem bark of *Acacia nilotica* against *Streptococcus mutans*

Solvent Used	MIC [MG/ML]
Ethanol	5.0
Petroleum ether	10.0

Table 5 shows the minimum inhibitory concentration of the extracts of *Acacia nilotica* against *Streptococcus mutans*. The MIC of ethanol extract was low [5mg/ml] as compared to petroleum ether extract [10 mg/ml]. The lower MIC of ethanol extract is an indication of high effectiveness of the extract.

DISCUSSION

Plant compounds are source of safer and more valuable therapeutic substitutes than synthetically created antimicrobial agents. Phytochemical analysis aids in the development of particular antimicrobial agents. Our study revealed that *Acacia nilotica* stem bark extracts contain alkaloids, saponins, cardiac glycosides, tannins, flavonoids and anthraquinones. These results are consistent with the findings by Tenguria et al. [2012][14]. Several plants which contain alkaloids, tannins, glycosides have been shown to possess antimicrobial activity against number of microorganisms as is investigated by Adebajo[1983][1]. In the present study antimicrobial activity of stem bark of *Acacia nilotica* was studied against *Streptococcus mutans*. It revealed high inhibitory activity against *Streptococcus mutans*. These results correlate with the studies of antimicrobial activity of stem bark of *Acacia nilotica* on various microorganisms by Banso [2009][2]. The extracts showed less antibacterial activity as compared to antibiotic gentamycin. However the difference of antibacterial activity of ethanol extract of stem bark of *Acacia nilotica* and antibiotic gentamycin is not significant. If safety and antibiotic resistance development issues are considered, it would still be beneficial to use *Acacia nilotica* for the management of dental caries.

Because *Streptococcus mutans* is generally considered as the main oral pathogen responsible for dental caries, the fact that *Acacia nilotica* inhibited the growth of *Streptococcus mutans* provides some scientific rationale for the use of this plant for the treatment of dental diseases.

CONCLUSION

The results of the present study show that *Acacia nilotica* is rich in phytochemicals. The phytochemical screening can serve as the basis for preparation of herbal monograph for proper identification and authentication of drug. A marked antibacterial activity was found against *Streptococcus mutans* in this study may be exploited in the treatment of dental caries caused by *Streptococcus mutans*.

REFERENCES

1. Adebajo ao, Adewumi CO, Esseini EE].Anti-infective agent of higher plants.Int.Syrup Med Plants [5th edn.]University of Ife, Nigeria; 1983pp.152-158.
2. Banso A.Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*.J.Med.Plants Res.2009; 3(2):082-085.
3. Chung JY, Choo JH, Lee MH, Hwang JK.Anticariogenic activity of macelignan isolated from *Myristica fragrans*(nutmeg) against *Streptococcus mutans*.Phytomedicine2006;13:261-6[cross Ref][Web of science][medicine].
4. Del WE.In vitro evaluation of peroxy radical scavenging capacity of water extract of *Acacia nilotica* (L)Afr.J. Biotechnol. 2009;8(7):1270-1272.
5. Devi A, Singh V, Bhatt A B.In vitro antibacterial activity of pomegranate and Daru(wild pomegranate) against dental plaque bacteria.Int J Pharma Sci 2011;3(4):182-184.
6. Gill LS.Ethanomedicinal uses of plants in Nigeria. University of Benin Press, Benin City, Nigeria.2009; pp.10—30.
7. Hema T A, Shiny M and Parvathy J.Antimicrobial activity of leaves of *Azima tetracantha* against clinical pathogens.Int J Pharma Sci 2012;4(4):317-319.
8. Loesche W.Role of *Streptococcus mutans* in human dental decay. Microbiology Review.1986;50:353-380.
9. Mann A, Gbate M, Umar A.Medicinal and economic plants.Jube Evans books and publication, Bida, Nigeria.2003;pp.160.
10. Meena PD, KaushikP, ShuklaS, SoniAK, KumarM, Kumar A.Anticancer and antimutagenic properties of *Acacia nilotica*(L)on Swiss albino mice.Asian Pac. J.Can.Prev.2006;7:627-632.
11. Park KM, YoujS, LeeHY, BaekNI, Hwang JK, Kuwanon G.An antibacterial agent from root bark of *Morus alba* against oral pathogens.J Ethnopharmacol.2003;84:181-5.[cross ref][web of science][Medline].
12. Prabhu GR, GnanamaniA, Sadulla S.Guaijaverin a plant flavonoid as potential antiplaque agent against *Streptococcus mutans*.J.Appl microbial.2006;101:487-95.
13. Raaman N.Phytochemical techniques, New India Publishing Company, New Delhi.2006;PP19-22.
14. Tenguria M, Chand P, Upadhyay R. Estimation of total polyphenolic content in aqueous and methanolic extract from the bark of *Acacia nilotica*. Int.J. Pharmacol.science Res.2012; 3(9):3458-3461.