ISSN 2278- 4136

ZDB-Number: 2668735-5

IC Journal No: 8192

Volume 1 Issue 5

Online Available at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

Preliminary Phytochemical Analysis and In Vitro Investigation of Antibacterial Activity of *Acacia nilotica* against Clinical Isolates

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Acacia nilotica commonly has been used in folk medicine to treat different diseases. In the present study, preliminary phytochemical analysis of ethanol and petroleum ether extract of stem bark of *Acacia nilotica* was carried out by using simple chemical tests. It revealed the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, cardiac glycosides and anthraquinone in both ethanol and petroleum ether extracts while fixed oils and fats, proteins and amino acids were absent. Antimicrobial activity of the extracts against clinical isolates was performed by agar diffusion method. It exhibited potent activity against all clinical isolates. The minimum inhibitory concentration for ethanol extract was 5 mg/ml while 10 mg/ml for petroleum ether extract. These results may be helpful for rationale use of this plant in the modern system of health care

Keyword: Phytochemical Analysis, *Acacia nilotica*, Agar Diffusion, Clinical Isolates, Health Care.

1. Introduction

Microbial infections are major public health problems in the developed countries. Antibiotics are used to treat these infections. Due to indiscriminate use of commercial antibiotics, the incidence of multiple antibiotic resistance in human pathogens is increasing. This has forced the scientists to search for new antimicrobial substances from various sources like medicinal plants. Medicinal plants constitute the main source of new pharmaceuticals and health care products[1] [Ivanona et al., 2005]. The use of traditional medicines is widespread in India[2] *[Jeyachandran, et al., 2007]*.

Acacia nilotica [Family-Mimosaceae] is a multipurpose plant. It is used for treatment of various diseases[3] *[Singh et al.,2009b]*. It serves as the source of polyphenols.The plant contains a profile of a variety of bioactive components[4] [Singh et al.,2009a].A number of medicinal properties have acute diarrhea[5] *[Gill; 1992]*.The bark of plant is used extensively for colds, bronchitis, diarrhea, bleeding piles and leucoderma[6] [Del; 2009].Pods and tender leaves are given to treat diarrhea and are also considered in folk medicine to treat diabetes mellitus[7] *[Gilani et al.,1999]*. The present study was conducted to screen the different phytochemicals present in the ethanol and petroleum ether extract of stem bark of *Acacia nilotica*. The aim of the current study was also to evaluate antibacterial activity of the extracts of stem bark of *Acacia nilotica* against the clinical isolates.

2. Material and Methods

a. 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.

b. Test Microorganisms

Various clinical isolates were obtained from V.M.Government Medical College, Solapur.The isolates were *Staphylococcus aureus ,E.coli,Proteus vulgaris, Proteus mirabilis,Salmonella paratyphi B, Klebsiella pneumoniae.*

c. 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^{0}c]. The extracts were kept in freeze until further use.

3. Phytochemical Analysis of Extract[8] *[Raaman, 2006]*

a. Detection of Alkaloids

1. **Mayer's test** –To a few milliliter of filtrate,a drop of Mayers reagent was 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 of Wagner's reagent were 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 was added.A prominent yellow precipitate indicates the test as positive.

b. 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.

c. Detection of Amino acids and proteins.

1. **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 colour in the ethanol layer indicates presence of proteins.
2. **Ninhydrin test** – Two drops of ninhydrin solution were added to 2 ml. of aqueous filtrate.A characterstic purple colour indicates the presence of aminoacids.

d. 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.

e. 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.

f. 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 colour develops, presence of flavonoids was inferred.

g. 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.

h. 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.

i. Detection of fixed oils and fats

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

3.1 Antibacterial activity of Plant Extract

Antibacterial activity of Plant Extract was determined by agar cup method. For this, fresh [overnight} isolated colony of the pathogen was suspended in sterile saline to get turbidity of 0.5 McFarland standard. o.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 by keeping in freeze for 20 minutes.10 % DMSO in one of the wells was used as negative control. After diffusion of extract the plates were incubated at 37 ^{0}c for 24 hours. Zones of inhibition were then measured in mm. For each extract, for each pathogen, three replicates were maintained.

3.2 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 suspension of each pathogen 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 inoculum. The tubes were incubated at 37^{0}c for 24 hours and checked for turbidity. Minimum inhibitory concentration was determined as highest dilution of the extract that showed no visible growth.

4. Results

4.1 Physical characteristics of extracts

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

Table 1 Physical characteristic of extracts

Sr. no.	Solvent used	physical characteristics		
		Colour	Odour	consistency
1	Ethanol	Brown	Agreeable	Dry crystalline
2	Petroleum ether	Dark brown	Agreeable	Dry crystalline

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

Table 2 Percentage yield of extracts

Sr. no.	Solvent used	Weight of dry powder [gms.]	Weight of dry extracts [gms.]	percentage yield
1	Ethanol	15	1.25	8.3
2	Petroleum ether	15	0.69	4.6

Table 3 shows the results of phytochemical analysis 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.

4.2 Phytochemical analysis of extracts

Table 3 Phytochemical analysis of extract

Sr.no.	Phytochemical	Ethanol extract	Petroleum ether extract
1	Alkaloids		
	Mayers test	-	-
	Wagner test	+	+
	Hagers test	-	-
2	Carbohydrates	+	+
3	Saponins	+	+
4	Proteins	-	-
5	Amino acids	-	-
6	Antraquinones	+	+
7	Tannins	+	+
8	Flavonoids	+	+
9	Fixed oils & fats	-	-
10	Cardiac glycosides	+	+

4.3 Antibacterial activity of extracts

Table 4 Antibacterial activity of extracts against various pathogens

Sr.no.	Name of pathogen	Zone of inhibition [mm]	
		Ethanol extract	Petroleum ether extract
1	*Staphylococcus aureus*	22	20
2	*Proteus vulgaris*	30	20
3	*Proteus mirabilis*	28	19
4	*Escherichia coli*	36	20
5	*Klebsiella pneumoniae*	*24*	20
6	*Salmonella paratyphi B*	*28*	22

Table 4 shows the results of antibacterial activity of ethanol and petroleum ether extracts of stem bark of *Acacia nilotica*. Both the extract inhibited the growth of all the pathogens used in this study. Ethanol extract showed more antibacterial activity as compared to petroleum ether extract. The highest zone of inhibition was observed of ethanol extract for *Escherichia coli followed by Proteus vulgaris.*

4.4 Determination of minimum inhibitory concentration of extract against various pathogens

Table 5 Minimum inhibitory concentration of the extracts against various pathogens

Sr. no.	Name of pathogen	MIC [mg./ml]	
		Ethanol extract	Petroleum ether extract
1	*Staphylococcus aureus*	5	10
2	*Proteus vulgaris*	2.5	10
3	*Proteus mirabilis*	2.5	10
4	*Escherichia coli*	2.5	10
5	*Klebsiella pneumoniae*	5	10
6	*Salmonella paratyphi B*	2.5	10

Table 5 shows the minimum inhibitory concentration of the extracts against various pathogens used in this study. The MIC of ethanol extract was low [2.5mg./ml] as compared to petroleum ether extract [10 mg/ml].The lower MIC is an indication of high effectiveness of extract. *Proteus vulgaris ,Proteus mirabilis, Escherichia coli and Salmonella paratyphi B showed* 2.5 mg/ml of MIC for ethanol extract while MIC of petroleum ether extract was high[10 mg/ml] for all the pathogens used in this study.

5. Discussion

The results of preliminary phytochemical analysis of ethanol and petroleum ether extract of stem bark of *Acacia nilotica* in the present study revealed the presence of alkaloids, saponins, cardiac glycosides, tannins. This finding is consistent with Banso[9] *[2009]*. In contrast, the present study showed presence of flavonoids in the ethanol extract of stem bark of *Acacia nilotica* which does not correlate with studies by Banso[9] *[2009]*.However the findings in present study correlate with preliminary analysis of stem bark ethanol extract by Siddiqui et al[10] *[2009]* who found the presence of flavonoids in the stem bark extract of *Acacia nilotica*. The antibacterial

potential of ethanol and petroleum ether extract of stem bark of *Acacia nilotica* was investigated against some of the pathogens like *Klebsiella pneumoniae Escherichia coli Staphylococcus aureus, Proteus vulgaris, Proteus mirabilis, Salmonella paratyphi B*. Both the extracts exhibited inhibitory action on the pathogens used in the present study. This finding correlates with reports of Dabur[11] et al *[2007]*. However ethanol extract showed greater activity as compared to corresponding petroleum ether extract. This may be due to stronger extraction capacity of active component responsible for antibacterial activity. The results of present study support the valuable use of *Acacia nilotica* in traditional medicines for treatment of infections caused by above tested bacteria.

6. Conclusion

The current study showed that *Acacia nilotica* is rich in phytochemicals. This plant showed potent antibacterial activity. This would be helpful to create awareness among people for taking control measures based on, herbal plants against infectious diseases. Herbal based medicines can be recommended alternate to antibiotics.

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