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EVALUATION OF PHYTOCONSTITUENTS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF ACACIA NILOTICA L.

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ABSTRACT

The study is described in which the ethanol and methanol extracts from *Acacia nilotica* L. bark were evaluated for their possible antibacterial and antioxidant activity. Preliminary phytochemical analysis of ethanolic and methanolic extracts was carried out by using simple chemical tests. Total phenolic content of both the extracts was determined by Folin-Lowry method. Ethanolic extract of *Acacia nilotica* L. was found to contain 307.54 ± 2.67 mg GAE/gm dry weight of phenolics and 496.42 ± 6.89 mg QE/gm dry weight of flavonoids. Antioxidant activity of both the extracts was evaluated by phosphomolybdenum method and DPPH radical scavenging method. Ethanolic extract demonstrated the highest DPPH radical scavenging activity of 71.8 ± 0.18 % at 1000µg/ml concentration. Both the extracts showed broad spectrum antibacterial activity. The study suggests that the plant has promising applications in the development of phytomedicines with antibacterial and antioxidant properties.

KEYWORDS: ***Acacia nilotica*** **L., Antioxidant, DPPH, antibacterial activity**

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INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of drugs have been isolated from natural sources[1]. Pharmaceutical importance of medicinal plants is due to specific constituents of secondary metabolites present in them[1]. World plant biodiversity is the largest source of herbal medicines and still about 60-80% world population rely on plant based medicines which are being used since the ages as traditional health care systems[2]. Though the recovery is slow, the therapeutic use of medicinal plants is becoming popular because they do not cause side effects and combat antibiotic resistant micro-organisms[3]. Antioxidants are agents which scavenge free radicals and prevent damage caused by reactive oxygen species (ROS) [4]. Several medicinal plants have been shown to exhibit potential antioxidant activity due to the presence of various phytochemicals, e.g. flavonoids, phenolics, tannins, carotenoids[5]. Cancer is the one of the most dreaded diseases of mankind and it is considered as an adversary of modernization and pattern of socio-economical life dominated by western medicine[6]. Herbal medicines seem to be important in prevention and treatment of cancer with minimum side effects. *Acacia* is pantropical and subtropical genus with species abundant throughout Australia, Asia, Africa and America. *Acacia nilotica* L. belonging to family Fabaceae is commonly known as Babool. It is commonly growing medium sized tree[7]. Mature trees can produce up to 2 – 4 kg seed in a good fruiting season[8]. Both young pods and mature seeds are edible. Pods are straight or slightly curved, 5 – 15 cm long on a pedicel, 0.5 – 1.2 cm wide with constrictions between the seeds giving the appearance of a string of pearls[8]. *Acacia nilotica* L. was found to have antimicrobial effects against *Xanthomonas malvacaerum*[9]. The methanolic extract of *Acacia nilotica* L. has shown to possess remarkable bactericidal properties against neuropathogenic *Escherichia coli*, MRSA and *Klebsiella* spp[10]. Various extracts of *Acacia nilotica* L. have shown to possess antioxidant activities[11, 8].

MATERIALS AND METHODS

1. Collection of plant material:

Bark of *Acacia nilotica* L. was collected from and around Modern College, Pune-05 campus and authenticated by Dr. Neeta Patil, Botany, Modern College, Pune. The bark was dried and powdered.

2. Preparation of extract:

Methanol extract was prepared according to method of Vaghasiya *et al*[12]. 10 gm dried powder of plant material was serially extracted first with petroleum ether and then with methanol. For ethanol extract, 10 gm air dried powder of plant material was extracted in 200 ml absolute ethanol by soxhlet extraction. The solvent was evaporated under vaccum and dry extract of plant material was dissolved in appropriate amount of respective solvent.

3. Qualitative phytochemical analysis of Acacia nilotica L.

Preliminary qualitative phytochemical analysis of ethanolic and methanolic extracts of *Acacia nilotica* L. was done to detect the presence of various phytoconstituents. The tests were performed to detect phytochemicals viz. Tannins ($FeCl_3$ test), Alkaloid (Dragendorff's test), Reducing sugars (Fehling's test), Protein (ninhydrin test), Steroids and triterpenoids (Salkowski test) and Saponin (froth test) [2].

4. Estimation of total Phenolic Content:

The total phenolic content in various extracts of *Acacia nilotica* L. was determined by method of Raghavendra[13] and Slinkard[14] with modifications. 10 µl of plant extract was added to 490 µl distilled water to which 2.5 ml Folin-Ciocalteau reagent (SRL) and 2 ml of 7.5% Na_2CO_3 was added. The reaction mixture was incubated for 90 min at room temperature and

absorbance was recorded at 750 nm. Gallic acid was used as standard.

5. Determination of total flavonoids:

Total Flavonoid content in various extracts was determined by the method of Raghavendra[13] and Zhishen[15] with some modifications. 50 µl of plant extract was added to 4950 µl Distilled water and was mixed with 0.3 ml of 5 % $NaNO_2$. This was incubated for 5 min at room temperature and 0.3 ml of 10 % $AlCl_3$ was added to the mixture. After 6 min of incubation 2 ml of 1M NaOH was added to the mixture followed by addition of 2.4 ml distilled water. Absorbance was recorded at 510 nm. Querscetin was used as standard.

6. Determination of total antioxidant capacity by phosphomolybdenum method:

The antioxidant activity of plant extracts was determined by method of Raghavendra[13] and Prieto[16] with some modifications. 10 µl plant extract was added to 290 µl distilled water was mixed with 3 ml of reagent solution (0.6 M Sulphuric acid, 28 mM Sodium phosphate and 4 mM Ammonium molybdate). The reaction mixture was incubated at 95° C for 90 min and after cooling, absorbance was recorded at 695 nm. L-Ascorbic acid was used as standard.

7. Evaluation of DPPH radical scavenging activity:

The free radical scavenging activity of plant extracts was determined by the method of Raghavendra[13] and Bracca[17] with some modifications. 10 µl plant extract of variable concentration (200-1000 µg/ml) was added to 290 µl of distilled water which is then mixed with 3 ml of 0.004% DPPH (1, 1-diphenyl-2-pecrylydrazyl) solution which is prepared in ethanol. Samples were incubated for 30 min in dark and absorbance was recorded at 517 nm. Standard compound L-Ascorbic acid was used for comparison. The % of DPPH radical scavenging activity was calculated as [(Ac-Ae)/Ac]*100, where

Ac - Absorbance of control

Ae - Absorbance of extract

8. Evaluation of antibacterial activity:

The antibacterial activity of *Acacia nilotica* L. was tested against *Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Klebsiella pneumoniae*. The antibacterial activity was assessed by seeding 0.1 ml of test bacterial culture (Optical density at 600 nm = 0.5) on Muller Hinton agar plates (Hi Media). 5 mm wells were made on agar surface with 5 mm cork borer to which 20 µl of each plant extract was added[18]. Plates were incubated at 37°C for 24 hrs and zone of inhibition was measured. Amoxicillin (1mg/ml) was used as positive control.

RESULT AND DISCUSSION

1. Qualitative phytochemical analysis of Acacia nilotica L.

Qualitative tests of extracts of *Acacia nilotica* L. were performed to detect the presence of various phytochemicals including Alkaloids, Tannins, Reducing sugars, Saponin, Steroids and Triterpenoids and Proteins. Bioactive compounds like Steroids, Alkaloids, Tannins, Reducing sugars, proteins were present in the solvent extracts. Alkaloids and tannins were present abundantly in both the extracts.

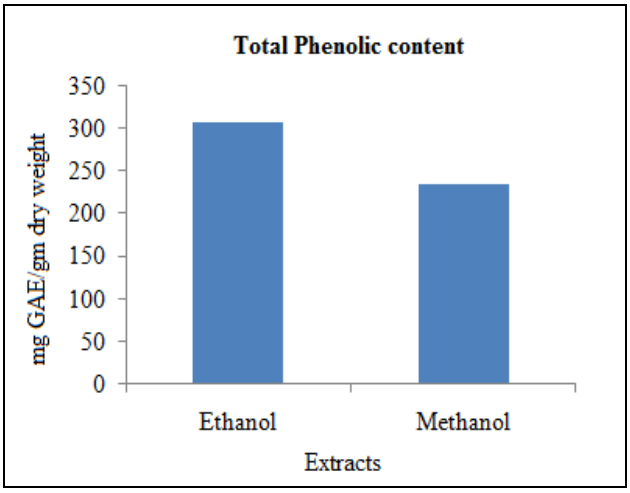
Table 1
Preliminary phytochemical screening

Phytoconstituents	*Acacia Nilotica* L.	
	Ethanol	Methanol
Alkaloids	+	+
Triterpenoids	+	+
Proteins	+	+
Tannins	+	+
Reducing sugars	+	+
Steroids	-	-
Saponin	+	+

2. *Total phenolic Estimation*

The antioxidant property of phenolics compound is mainly because of their redox properties which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides[18]. Total phenolic content of *Acacia nilotica* L. was assayed by Folin-Ciocalteau method. The content of phenolics compounds was determined from regression equation of calibration curve (y=0.022x–0.043, R^2=0.993) and expressed as Gallic acid equivalents (GAE). Among the 2 extracts evaluated, ethanolic extract of *Acacia nilotica* L. showed highest amount of phenolics i.e. 307.54 ± 2.67 mg GAE/gm dry weight followed by a methanolic extract with phenolic content of 234.19 ± 5.17 mg GAE/gm dry weight of plant material.

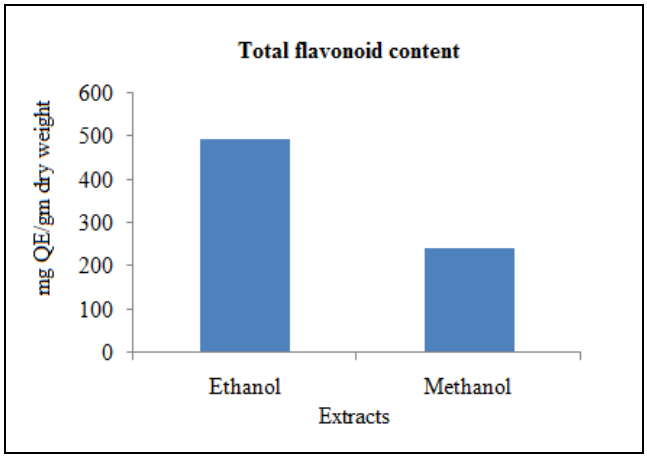
Graph 1
Total Phenolic content



3. *Total flavonoid Estimation*

Flavonoids are potent antioxidants *in vitro* and therefore are of main interest as they are involved in protection against cardiovascular diseases[13]. The content of flavonoids was determined from regression equation (y=0.003x+0.007, R^2=0.997) of calibration curve and total flavonoid content was expressed in Querscetin equivalents (QE). Among both the extracts tested for total flavonoid content, ethanolic extract showed highest amount of flavonoids which was 496.42 ± 6.89 mg QE/gm dry weight followed by methanolic extract with flavonoid content of 243.16 ± 8.46 mg QE/gm dry weight of plant material.

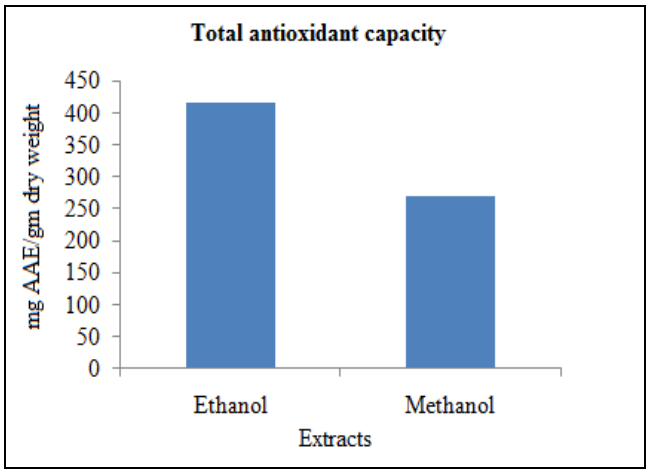
Graph 2
Total flavonoid content



4. Estimation of total antioxidant capacity:

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm[13]. The total antioxidant capacity was determined by the regression equation of calibration curve (y = 0.002x+0.023, R^2=0.981) and expressed as Ascorbic acid equivalents (AAE). Among both the extracts evaluated for their total antioxidant capacity, ethanolic extract of *Acacia nilotica* L. showed highest antioxidant activity i.e. 416.60 ± 8.28 mg AAE/gm dry weight and methanolic extract was found to possess significant antioxidant activity of 268.69 ± 5.96 mg AAE/gm dry weight of plant material.

Graph 3
Total antioxidant capacity

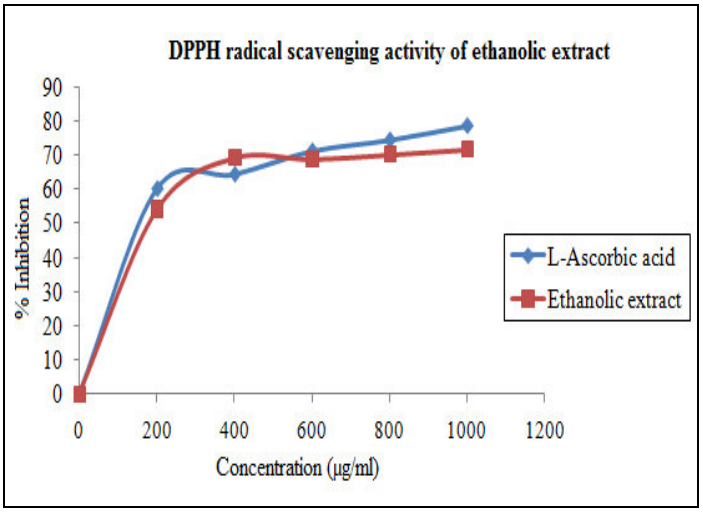


5. Estimation of DPPH radical scavenging activity

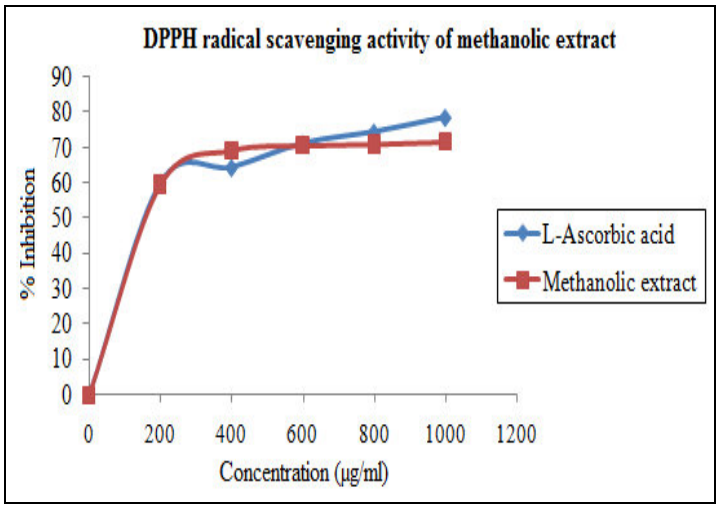
The concentration dependent antioxidant activity of ethanolic and metanolic extracts of *Acacia nilotica* L. was assayed by using 1, 1-diphenyl-2-pecrylydrazyl (DPPH) method. The scavenging effect was found to increase with increase in concentration. The highest % inhibition or radical scavenging activity was shown by an ethanolic extract at concentration 1000 µg/ml and was 71.8 ± 0.18 % which was comparable to activity of standard compound L-

Ascorbic acid i.e. 78.48 ± 2.11 % at same concentration. Methanolic extract also showed the promising radical scavenging activity of 71.55 ± 0.10 % at the same concentration. The ability of free radical scavenging probably is one of the mechanism by which the herbal medicines exhibit their antioxidant capacity.

Graph 4
DPPH radical scavenging activity of ethanolic extract



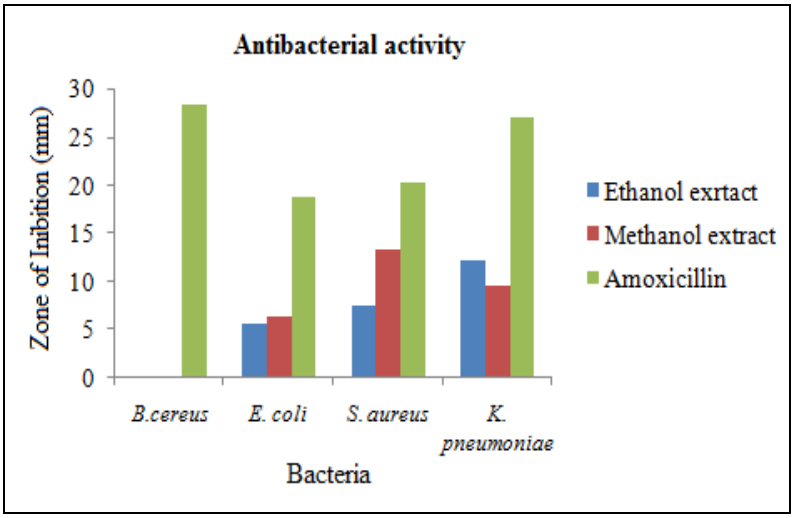
Graph 5
DPPH radical scavenging activity of methanolic extract



6. Evaluation of antibacterial activity:

Both the extracts of *Acacia nilotica* L. were tested for their antibacterial activity against Gram positive and Gram negative bacteria including *Staphylococcus aureus*, *Bacillus cereus, Escherichia coli*, and *Klebsiella pneumoniae*. The antimicrobial activity was determined by agar well diffusion method and zone of inhibition was recorded. Ethanolic and methanolic extracts of *Acacia nilotica* L. were found to be effective against all the tested bacteria except *Bacillus cereus*. *Bacillus cereus* was found to be resistant to both the extracts. Standard drug Amoxicillin was found to be very effective against all the tested bacteria.

Graph 6
Evaluation of Antibacterial activity of Acacia nilotica L.



CONCLUSION

The data obtained from the present study reveals the presence of various phytoconstituents like tannins, alkaloids, reducing sugars, proteins, saponin etc. in 2 different solvent extracts *Acacia nilotica* L. Total phenolic content and total flavonoids content was higher in *Acacia nilotica* L. ethanolic extract than methanolic extract. Ethanolic extract of *Acacia nilotica* L. contains an effective antioxidant fraction that has been confirmed by phosphomolybdenum method and by DPPH radical scavenging activity. Antimicrobial activity of plant extract has been shown that *Acacia nilotica* L. has a broad spectrum of activity which can be used as leads in developing the novel therapeutic bioactive agents. From the above investigation it can be recommended that *Acacia nilotica* L. could be used as easily accessible natural source, which can be used to aid therapy for the diseases caused by free radicals. Further studies are required to assess the toxicity and safety for utilization of antioxidant components of *Acacia nilotica* L. in different pharmaceutical formulations.

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