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EVALUATION OF PHYTOCHEMICAL, *IN VITRO* ANTIBACTERIAL AND CYTOTOXIC PROPERTIES OF ETHANOL EXTRACT OF *ACACIA NILOTICA* (L) LEAVES

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Abstract:
Acacia nilotica L. commonly has been used in folk medicine to treat different diseases. The aim of the present study is to evaluate the presence of nutrients and demonstrate the antibacterial and cytotoxic properties of the correspondence plant leaves extract. Preliminary phytochemical analysis of ethanol extract of leaves of A. nilotica was carried out by using simple chemical tests. Antimicrobial activity of the extract against diarrheal bacteria was performed by disc diffusion method. The cytotoxicity was determined by brine shrimp lethality bioassay. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, cardiac glycosides, anthraquinone, steroid, triterpenes, terpenoid, gum, amino acids and proteins but fixed oils and fat was absent. It exhibited potent activity against all bacteria. The minimum inhibitory concentration (MIC) for the extract was 128µg/ml against both Shigella boydii and Vibrio cholerae. The extract showed significant toxicity to the brine shrimp nauplii giving LC_{50} was 395.581 ppm. The plant leaves extract might be used as a good source of nutrient. It also could be used as antibacterial agent in the future as herbal medicine. Further study on different solvent extracts would be carried out to elucidate the active principles for its outmost activity.

Keywords: *Phytochemical, Acacia nilotica, antibacterial, cytotoxicity, MIC.*

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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 resistances in human pathogens is increasing. This has forced the scientists to search for new antimicrobial substances from various natural sources like medicinal plants. Medicinal plants constitute the main source of new pharmaceuticals and health care products [1]. The use of traditional medicines is widespread in India [2]. *Acacia nilotica* [Family-Mimosaceae] is a multipurpose plant. It is used for treatment of various diseases [3]. It serves as the source of polyphenols. The plant contains a profile of a variety of bioactive components [4]. The bark of plant is used extensively for colds, bronchitis, diarrhea, bleeding piles and leucoderma [5]. Pods and tender leaves are given to treat diarrhea and are also considered in folk medicine to treat diabetes mellitus [6].The plant has been shown to exhibit antibacterial [7], anti-inflammatory [8], antiplatelet aggregatory activity [9], cestocidal activity [10], antibacterial effects [11], spasmogenic, vasoconstrictor actions [12], antihypertensive, antispasmodic activities [13], inhibitory effect against hepatitis C virus [14] and cytotoxic activity [15]. The present study was conducted to screen the different phytochemicals present in the ethanol extract of leaves of *A. nilotica.* The aim of the current study was also to evaluate antibacterial and cytotoxic activities of the extracts of leaves of *A. nilotica* against the diarrhoeal bacteria.

MATERIALS AND METHODS:

The leaves of the plant *A. nilotica* were collected from Rajshahi University campus, Bangladesh. It was identified and authenticated in the department of Botany, Rajshahi University, Bangladesh.

Test microorganisms

The test microorganisms will obviously depend greatly on the purpose of the investigation. The pure cultures were collected from the Institute of Biological Science, Department of Pharmacy, University of Rajshahi, and Environmental Microbiology Lab (ICDDRB), Mahakhali, Dhaka, Bangladesh. The bacteria were used for the study of antibacterial activity as follows, *Escherichia coli, Shigella sonnei, Shigella dysenteriae, Shigella shiga, Shigella boydii, shigella flexneri* and *Vibrio cholera.*

Preparation of plant extract

Fresh leaves parts of the plant materials were washed under running tap water and air dried for about one week and then they were homogenized to fine powder and were stored in airtight bottle. The powder of leaves materials (100gm) was extracted with 100ml ethanol using conical flask in a shaking incubator at 28°C for two days. The ethanol extract was filtered and evaporated until dryness. The extract was stored at 4°C until for further use.

Phytochemical analysis of extract

The following tests were performed for identifying different chemical groups [16].

Test for gums

5ml solution of the extract was taken and then Molisch reagent and sulphuric acid were added to identify gums.

Test for Carbohydrates

Molisch's test: A few drops of molisch reagent was added to a little quantity of extract in a test tube and a small quantity of concentrated sulphuric acid was allowed to run down the side of the test tube to form a violet layer at the interface indicated the presence of carbohydrates.

Fehlings Test: To 2ml of extract, 5ml of a mixture of Fehling's solution A and B in the ratio of 1:1 was added and the mixture boiled for few minutes in water bath. A brick-red precipitate indicated the presence of free reducing sugar.

Test for Free Anthraquinones

Borntrager's test: Small portion of the extract was mixed with 10ml of benzene and filtered. Then 5ml of 10% of ammonia solution was added to the filtrate and stirred. The production of a pink-red or violet color indicated the presence of free anthraquinones.

Test for Combined Anthraquinones

Sample was boiled with 5ml of 10% hydrochloric acid for 3 minutes. This would hydrolyze the glycosides to yield glycones which are soluble in hot water only. The solution was filtered at hot condition. The filtrate was cooled and extracted with 5ml of benzene. The benzene layer was filtered off and shaken gently with half its volume of 10% ammonia solution. A rose- pink or a cherry red color indicated combined anthracene by presence of free anthraquinones.

Test for Cardiac Glycosides

Kella-Killiani Test: Extract was dissolved in glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45° and 1ml of concentrated sulphuric acid was added down the side. Purple ring color at the interface indicated cardiac glycosides.

Test for saponins

Frothing test: Small quantity of the extract was dissolved in 10ml of distilled water. This was then

shaken vigorously for 30 seconds and was allowed to stand for 30 minutes. A honey comb foam formed for more than 30 minutes indicated the presence of saponins.

Test for Steroid and Triterpenes
Lieberman-Burchards test: Equal volume of acetic anhydride was added to the extract. One milliliter of concentrated sulphuric acid was added down the side of the tube. The color change was observed immediately and later. Red, pink or purple colour indicated the presence of triterpenes, while blue or blue-green indicated steroids.

Tests for Flavonoids
Shinoda Test: About 0.5g of extract was dissolved in 2ml of 50% methanol in the tube. Metallic magnesium and four to five drops of conc. hydrochloric acid was added. A red or orange color indicates the presences of flavanoicaglycones.

Test for Tannins/Phenol
Lead sub-acetate test: Three drops of lead-sub acetate solution were added to a solution of the extract. A colored precipitate indicated that tannins are present.
Ferric chloride test: About 0.5ml of extract was dissolved in 10ml of distilled water, and then filtered. A few drops of ferric chloride solution were added to the filtrate. Formation of a blue-black precipitate indicated the presence of hydrolysable tannins and green precipitate indicated that of condensed tannin.

Test for Alkaloids
Meyer's Test: A few drops of the Meyers reagent was added to an aliquot of the extract in a test tube Cream precipitateindicated the presence of alkaloids.
Dragendoffs test: A few drops of this reagent were added to the extract. A rose red precipitate indicated the presence of alkaloids.
Wagners Test: A few drops of this reagent were added to a small amount of the extract. A whitish precipitate indicated the presence of alkaloids.
Picric acid test: A few drops of 1% picric acid solution were added to the extract and a yellow colored solution indicated the presence of alkaloids.

Detection of Amino acids and proteins
The extract (100mg) was dissolved in 10ml 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 the presence of proteins.

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

Test for terpenoids
Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated sulphuric acid was added and heated for about 2 minutes. A grayish color indicated the presence of terpenoid.

Antimicrobial assay
The antimicrobial activity was investigated using disc diffusion assay. Reference microorganisms from the stock were streaked onto nutrient agarplates and the inoculated plates were incubatedovernight at 37°C. Using a sterile loop, small portionof the subculture was transferred into test tubecontaining nutrient broth and incubated (2-4h) at37°C until the growth reached log phase. Nutrientagar media seeded with standard inoculum suspensionwas poured in petri-dishes (7mm diameter) and allowed tosolidify. Measured amount of each test samples were dissolved in specific volume ofsolvent (chloroform or methanol) to obtain thedesired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank petri-dish under thelaminar hood. Then discs were soaked with solutionsof test samples and dried. Discs impregnatedwith extract and control (solventchloroform or methanol) discs were placed on thepetri-dishes with sterile forceps and gently pressedto ensure contact with the inoculated agar surface.Finally the inoculated plates were incubated at 37° Cfor 24h and the zone of inhibition was measured in millimeters.

Determination of MIC (minimum inhibitory concentration)
Tube dilution method was done to determine minimum inhibitory concentration of the extracts. A series of two fold dilutions of extracts ranging from 10mg/ml to 0.3 mg/ml were made in Muller Hinton broth. 0.1ml 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°C for 24 hours and checked for turbidity. Minimum inhibitory concentration was determined ashighest dilution of the extract that showed novisible growth.

Cytotoxicity test

The brine shrimps used for cytotoxicity test were obtained by hatching 5mg of eggs of *Artemia salina* in natural seawater after incubation at about 29°C for 24h. The larvae (nauplii) were allowed another24 h in seawater to ensure survival and maturity before use. Five doses of plant extract (100, 200, 400, 600 and 800 ppm) in 5% DMSO and/or seawater was tested. Each extract preparation was dispensed into clean test tubes in 10ml volumes and tested in duplicates. The concentration of DMSO in the vials was kept below 10µl/ml. For control, same procedure was followed except test samples. Aftermarking the test tubes properly, 10 living shrimps were added to each of the 6 vials with the help of a pasteur pipette. The test tube containing the sample and control were then incubated at 29°C for24h in a water bath, after which each tube was examined and the surviving nauplii

counted. Fromthis, the percentage of mortality was calculated at each concentration.

RESULTS:

Phytochemical analysis of extract

The results of different chemical tests for the crude ethanolic extracts are shown in Table 1. The leaves extract of *A. nilotica* showed the presence of alkaloid, carbohydrates, saponins, tannins, flavonoids, cardiac glycosides, anthraquinone, steroid and triterpenes, terpenoid, gum, amino acids and proteins in the extract but fixed oils and fat was absent.

Antimicrobial activity and MIC of the extracts against diarrheal bacteria

The ethanolic extract of leaves of *A. nilotica* was showed significant activity against bacteria. From this experimental study we can summarize the activity of this extract as a potent antibacterial agent. It is a preliminary investigation. Further study should be done for more scientific evidence (Table 2). The MIC of ethanol extract was low 16µg/ml in *S. sonnei*. The lower MIC is an indication of high effectiveness of extract. *E. coli* 32 µg/ml, *S. dysenteriae* 64 µg/ml, *S. shiga* 32 µg/ml, *S. boydii* 128 µg/ml, *S. flexneri* 64 µg/ml and *V. cholerae* 128 µg/ml, respectively for ethanol extract of *A. nilotica* leaves. The MIC of extract was high 128 µg/ml for *S. boydii* & *V. cholera* for all the pathogens used in this study. No zone showed by the control.

Table1: Phytochemical analysis of *A. nilotica* leaves extract

Tests	Ethanol
Alkaloids	+
Carbohydrate	+
Anthraquinones (Free state)	+
Anthraquinones (Combined state)	+
Cardiac Glycosides	+
Saponins	+
Steroid & Triterpenes	+
Flavonoids	+
Phenol/Tannins	+
Amino acid & protein	+
Terpenoid	+
Fixed oil & fat	-
Gum	+

(+=Present, - = Absent)

Table 2: *In Vitro* antibacterial activity of ethanol extract of *A. nilotica* leaves with their MIC against the diarrheal bacteria

Sl. no.	Name of bacteria	Zone of inhibition(mm)	MIC(µg/ml)
		Ethanol extract	Ethanol extract
1	*E. coli*	8.00	32
2	*S. dysenteriae*	8.67	64
3	*S. shiga*	8.33	32
4	*S. sonnei*	9.67	16
5	*S. boydii*	10.33	128
6	*S. flexneri*	8.00	64
7	*V. cholerae*	8.67	128

MIC= minimum inhibitory concentration

Table 3: LC_{50} values, 95%, regression equations and χ^2 values (along with their df) of the ethanol extract of *A. nilotica* leaves against *A. salina* nauplii

Ethanol Extract	Exposure (h)	Concentration (ppm)	Log concentration	No. of kill nauplii	% mortality	Regression equations	LC_{50} (ppm)
A. nilotica leaves	24	800.000	2.903	8	26.667	Y = 0.601 + 1.706X	395.581
		600.000	2.778	6	20.000		
		400.000	2.602	4	13.333		
		200.000	2.301	3	10.000		
		100.000	2.000	2	6.667		

Cytotoxic activity

Table 3 shows brine shrimp lethality bioassay, the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. From the plot of percent mortality versus log concentration on the graph paper LC_{50}.

DISCUSSION:

The results of preliminary phytochemical analysis of ethanol extract of leaves of *A .nilotica* in the present study revealed the presence of alkaloids, saponins, cardiac glycosides, tannins. This finding is consistent with another study [17]. In contrast, the present study showed presence of flavonoids in the ethanol extract of leaves of *A. nilotica* which does not correlate with the studies [17]. However the findings in present studycorrelate with preliminary analysis of stem barkethanol extract [18], who found the presence of flavonoids in the stem bark extract of *A. nilotica*. The antibacterial potential of ethanol extract of leaves of *A. nilotica* was investigated against some of the pathogens like *E. coli, S. dysenteriae, S.shiga, S. boydii, S. sonnei, S. flexneri & V .cholerae.* All the extracts exhibited inhibitory action on the pathogens used in the present study. This finding correlates with reports of previous study [19]. The cytotoxic activity of the ethanol extract of dried leaves of *A. nilotica* was tested by using brineshrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. Brineshrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor [20]. The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that theextract may contain antitumor, antibacterial or pesticidal compounds. This may be due to stronger extraction capacity of activecomponent responsible for antibacterial and cytotoxic activities.The results of present study support the valuable use of *A. nilotica* in traditional medicines fortreatment of infections caused by above tested diarrheal bacteria.

CONCLUSION:

The current study showed that *A. nilotica* is rich in phytochemicals. This plant leaves extract showed potential antibacterial and cytotoxic properties. This would be helpful tocreate awareness among people for taking control measures based on, herbal plants against infectious diseases including diarrhea. Herbal based medicines canbe recommended alternate to antibiotics.

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Authors'contributions

Md. Belal Uddin provides the conception design and conduction of the research. Mrityunjoy Das carefully participated for the acquisition, analysis and interpretation of data. M. Sohanur Rahman and Md. Maniruzzaman participated to the critical revision. All authors read and approved the final manuscript. Finally Md. Belal Uddin supervised the whole critical submission process.

Conflict of interests

The authors declare that they have no competing interests.

REFERENCES:

1.Ivanona D, Gerova D, Chervenkov T and Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants.J. Ethnophrmacol., 2005;96:145-150.

2. Jeyachandran R and Mahesh A. Enumatration of antidiabetic herbal flora of Tamilnadu. Res. J. Med. Plant.2007;1:144-148.

3. Singh BN, Singh BR, Singh BK andSingh HB. Potential chemo-prevention of Nnitrosodiethylamine induced hepatocarcinogenesis by polyphenolics from *Acacia nilotica*bark.Chem-Biol. Interact,2009; 181:20-28.

4.Singh BN, Singh BR, Singh RL, Prakash D, Sarma BK and Singh HB. Antioxidant and antiquorum sensing activities of green pod of *Acacaia nilotica* L. Food. Chem. Toxicol.,2009; 47:778- 786.

5. Del WE. *In Vitro* evaluation of peroxyl radical scavenging capacity of water extract of *Acacia nilotica* (L). Afr. J. Biotechnol.,2009; 7:1270-1272.

6.Ghani A 1998. Medicinal Plants of Bangladesh, 1st ed. Asiatic Society Dhaka, 1st edition; p13.

7.Abd E, Nabi OM, Reisinger EC, Reinthaler FF, Still F, Eibel U and Krejs GJ 1992. Antimicrobial activity of *Acacia nilotica* (L.) Willd. Ex Del. Var. nilotica (Mimosaceae).J. Ethnopharmacol.,37:77-79.

8. Dafallah AA and Al-Mustafa Z. Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*, Am. J. Chin. Med., 1996;3-4:263-269.

9. Shah BH, Safdar B, Virani SS, Nawaz Z, Saeed SA and Gilani AH. The antiplatelet aggregatory activity of *Acacia nilotica* is due to blockade of calcium influx through membrane calcium channels. Gen. Pharmacol.,1997;2: 251-255.

10. Ghosh NK, Babu SPS, Sukuland NC, Ito A. Cestocidal activity of Acacia auriculiformis. J. Helmintho l.,1996;70:171-172.

11. Sotohy SA, Sayed AN and Ahmed MM. Effect of tannin-rich plant (*Accacia nilotica*) on some nutritional and bacteriological parameters ingoats. Deutsche.Tierarztliche.Wochenschrift.1997;,104: 432–435.

12. Amos S, Akah PA, Odukwe CJ, Camaniel KS and Wambede C.The pharmacological effects of an aqueous extract from Acacia nilotica seeds.Phytother. Res.,1999;13: 683-685.

13. Gilani AH,Shaheen F, Zaman M, Janbaz KH, Shah BH and Akhtar MS. Studies on antihypertensive and antispasmodic activities of methanol extract of *Acacia nilotica*. Pods.,1999; 13:665-669.

14. Hussein G, Miyashiro H, Nakamura N, Hattori M, Kakiuchi N and Shimotohno K. Inhibitory effect of Sudanese medicinal plant extracts on hepatitis C virus protease. Phytother. Res.,2000;14: 510-516.

15. Tezuka Y, Honda K, and Banskota AB. Three new cytotoxic saponins from the fruits of *Acacia concinna*, a medicinal plant collected in Myanmar. J. Nat. Prod.,2000;63: 1658-1664.

16. Raaman N. Phytochemical techniques, New Delhi, 2006; pp.19-22.

17. Banso A. Phytochemical and antibacterial investigation of bark extracts of Acacia nilotica. J. Med. Plants Res.,2009; 2:82-85.

18. Siddiqui MB and Husain W.Traditional treatment of diarrhea and dysentery through herbal drug in rural India. Fitoterpia.,1991; 62:325-529.

19. Dabur R, Gupta A, Mandal TK, Singh DD, Bajpai V, Gurav AM and Lavakar GS 2007. Antbacterial activity of some Indian medicinal plants. Afr. J. Trad. CAM.,3:313-318.

20. Anderson JE, Chang CJ and McLaughlin JL. Bioactive components of Allamanda schottii. J. Nat. Prod.,1998;51: 307-308