

Phytochemical and antibacterial investigation of *Vachellia nilotica* (bark and leaves) under agro-ecological regions of Punjab

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ABSTRACT

The present study was based on different pharmacological activities i-e phytochemical, antibacterial, proximal analysis and mineral analysis of plant specie *vachellia nilotica* (leaves and bark) collected from two regions of Punjab, i.e. Bahawalpur and Rahim Yar Khan. The phytochemical analysis of *vachellia nilotica* show the existence of flavonoids, Alkaloids, Amino acids, Tannins, saponins, proteins and carbohydrates. The proximal analysis shows the presence of crude fat, crude protein, moisture content, dry matter and Ash. Antibacterial activity was studied by measuring the Zone of inhibition in (millimeters) formed around the disc.

Keywords: Medicinal plants, Species, Antibacteria

INTRODUCTION

Medicinal plants are used as a source of comfort from sickness that can be discovered in India china and the near east. In the present day, plants are used as a source of phytochemical, pharmacological and antitoxins in ethno-medicine worldwide (Saeed et al., 2022; Safeer et al., 2022; Umair et al., 2022). The half population of the world depends on plant extracts, At one time more than 30% of plant species for health care or further plants were utilized as medicinal occasion, many are used in the Ayurveda system of medicine in ancient times. In India herbal extracts were used in the conventional organization of medicine (Umair et al., 2017; Amjad et al., 2020; Umair, 2020; Naz et al., 2022).

These medicinal plants assemble a certain physiological activity in the human body due to the presence of chemically active substances. These plants have most important bioactive components, phenolic, tannin, flavonoid and alkaloid compounds. The infection has expanded to a considerable level and antibiotic resistance implied enhances the therapeutic complication within recent years. Higher medicinal plant products may possess new sources of antimicrobial agents that have the possibility of unique mechanisms (Thirumurugan et al., 2010).

MATERIALS AND METHODS

Collection of plant material

Fresh leaves and bark of medicinal plant, *Vachellia Nilotic* were collected from two regions of Pakistan, province Punjab, city Bahawalpur, area cholistan desert (Altaf, 2022) and Rahim Yar Khan village.

Plant extract Preparation

The leaves and bark of *Vachellia nilotica* was washed from tap water very gently. It was then dried in the shade and changed into powder in the electronic machine. 20gm of powder was then extracted in 1000 ml of methanol, mix well with a stirrer, places it for a week and then filtered with Whatman filter paper. The solvent was evaporated by evaporation at normal surrounding

temperature. The obtained residues were weighed and kept in bottles and were used for further phytochemical and biological screening studies. Phytochemical analysis of the extract The methods described by (Harborne, 1998) with few changes were used to test for the presence of the active ingredients in the test sample. Test for steroids A 10 ml of chloroform extract of the test plant leaves was evaporated to a dry mass and the mass dissolved in 0.5 ml of chloroform. Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added. A blue or green color or a mixture of these two shades was regarded as positive for the presence of steroidal compounds.

Test for terpenoids

The presence of terpenoids was determined as described for steroids except that red, pink or violet colour indicates the presence of terpenoids. Test for tannins

1 cm³ of freshly prepared 10% KOH was added to 1 cm³ of the extract. A dirty white precipitate indicated the presence of tannins. The powdered stem bark of the test plant (1.0 g) was weighed into a beaker and 10 ml of distilled water was added. The mixture was boiled for five minutes. Two drops of 5% FeCl₃ were then added. Production of greenish precipitate indicated the presence of tannins.

Test for flavonoids

A small piece of magnesium ribbon was added to ethanolic extract of the plant material, this was followed by the dropwise addition of concentrated hydrochloric acid. Colours varying from orange to red indicated flavones, red to crimson indicated flavonols, and crimson to magenta indicated flavanones (Harborne, 1978).

Test for alkaloids

The extract of the plant stem bark sample (0.5 g) was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. The turbidity of the extract filtrate on the addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract (Harborne, 1998).

Test for saponins

Stem bark of the test plant was ground into powder form and 0.5 g of the powdered stem bark was introduced into a tube containing 5.0 ml of distilled water, the mixture was vigorously shaken for 2 min, formation of froth indicated the presence of saponins.

Test for glycosides

Coarsely powdered stem bark (1 g) was added into two separate beakers. To one of the beakers was added 5 ml of dilute sulphuric acid while 5 ml of water was added to the other beaker. The two beakers were heated for 3 - 5 min and the contents were filtered into labeled test tubes. The filtrate was made alkaline with 5% sodium hydroxide and heated with Fehling's solution for 3 min. The presence of a reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides (Harborne, 1998).

Antimicrobial assay

Take 100ml distilled water 8gm Broth was dissolved and put in test tubes and autoclaved at 121-degree centigrade for 20 minutes. After that inoculated bacteria in it and then incubate in a shaker incubator for 48 hours at 25 degree centigrade. We take five types of Bacteria.

Take 14.5 gm of nutrient Agar in glass bottle and dissolve in 500ml water, Shake the bottle in round pattern in a way that its bottom touches the ground level of shelf to avoid bubble formation. Put 20ml of nutrient agar in 1 petridish. 1 gram of plant extract was absorbed in 200 ml of solvent (H₂O) in a beaker(for each sample). Plastic petridishes were wrapped in a paper and air tighted with plastic bag and autoclaved / sterilized at 121 degree centigrade.

0.5 cm disc was made from filter paper (whattman), it was also autoclaved/sterilized. Sterilization of filter paper +petridishes was done at 121 degree centigrade for almost 20 minutes. We poured bacteria in 30 petridishes and placed in incubator for 24 hours at 25 degree centigrade, and note the inhibition zone of bacteria.

Table 1: Phytochemical analysis of bark and leaves extract.

ACTIVE PRINCIPLE	EXTRACT
Steroids	-
Terpenoids	+
Tannins	+
Flavonoids	-
Alkaloids	+
Saponins	+
Glycosides	+
+=present	
-=Absent	

Table 2: Antimicrobial activities of Leaves bark extract.

Mean diameter of zone of inhibition (mm) $\pm$ SDConcentration (mg/ml)	Pa	kp	Sa	Pm	Ec
5	0	0	0	0	0
10	0	0	0	0	0
15	2	7.5 $\pm$ 0.1	10.0 $\pm$ 0.01	6.5 $\pm$ 0.02	0
20	10.5 $\pm$ 0.05	14.0 $\pm$ 0.05	8.5 $\pm$ 0.02	5.0 $\pm$ 0.04	0
25	13.0 $\pm$ 0.1	16.5 $\pm$ 0.2	11.5 $\pm$ 0.02	9.5 $\pm$ 0.01	5.0 $\pm$ 0.01
30	15.5 $\pm$ 0.02	18.0 $\pm$ 0.05	15.0 $\pm$ 0.01	13.5 $\pm$ 0.1	8.5 $\pm$ 0.02

Pa = Pseudomonas Aeriginosa

Kp = Klebsiella Pneumonia

Sa = Staphylococcus Aureus

Pm = proteus Mirabella

Es = Eschercia coli

Anti Bacterial Analysis

Determination of minimum Inhibitory concentration (MIC)

Many concentrations of plant extract ranging between 10 and 60 mg/ml were introduced into different test tubes each tube was immunized with stout cultures of *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Proteus mirabilia*, *Eschericia coli* diluted to give a final concentration of 10 cells per ml. The tubes were inoculated for 24 hours at 37 degree centigrade. The least concentration of the plant extract that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the MIC in each case (Andrews, 2001).

Determination of minimum bactericidal concentration (MBC)

After culturing the test organisms separately in nutrient broth containing various concentration of the stem bark extract of the plant, the broth was inoculated onto freshly prepared agar plates to assay for the bactericidal effect. The culture was incubated at 37°C for 24 h. The lowest concentration of the plant extract that does not yield any colony growth on the solid medium after the incubation period was regarded as MBC (Taylor et al., 1983).

Table 3: Minimum inhibitory concentration (MIC) of stem bark extract.

Organism	MIC (mg / ml)
<i>Pseudomonas Aeriginosa</i>	40
<i>Klebsiella Pneumonia</i>	35
<i>Staphylococcus Aureus</i>	40
<i>Proteus Mirabella</i>	45
<i>Eschericia coli</i>	50

Table 4: Minimum bactericidal concentration (MBC) of stem bark extract .

Organism	MBC (mg/ml)
<i>Pseudomonas Aeriginosa</i>	45
<i>Klebsiella Pneumonia</i>	35
<i>Staphylococcus Aureus</i>	40
<i>Proteus Mirabella</i>	50
<i>Eschericia coli</i>	60

Purpose

Determination of Micronutrients and Heavy Metals in plants. Equipment's /Apparatus Required:
Atomic Absorption spectrophotometer..

Tubes for Digestion

Block for Digestion Hood fumes.

Volumetric type flask Pipette

Grinder Drying oven

Weighing balance.

Reagents

Perchloric acid and Nitric Acid mixture (1:2): Add 500ml (70%) perchloric acid in 1000 ml HNO₃(69-71%), mix well, cool and store in an amber glass bottle. Standard solutions for the

desired micronutrients.

Process

Add 25 ml Nitric acid+ perchloric acid (2:1) solution in a weighted 1 gm sample and, Digest at 150- 175 degree centigrade when the clear liquid obtained. with distilled water and filter made the volume 50ml. Prepare the standard following.

Zinc and Cu 0,0.25,0.50,1.00 ppm*

Iron and Manganese 0,0.5,1.0,2.0 ppm*

According to the sample nature Values may be Varied. Examined on atomic absorption spectrophotometer and used the following lamp.

Readings

Micronutrients (parts per million) = Reading *50 6) precautions/Safety :needs \6.1 Always run a blank for accurate results.

Table 5: Mineral Analysis

Plant part	Zn (ppm)	Cu(ppm)	Fe(ppm)	Mn(ppm)
Bark Bwp	60.4	62.6	661.7	17.4
Leaves Bwp	74.4	66.6	416.0	26.5
Bark Ryk	89.8	79.2	180.3	9.0
Leaves Ryk	79.8	77.2	485.3	26.8

RESULTS AND DISCUSSION

Three bacterial species viz, *Escherchia coli* TCC 729, *Agrobacterium tumefaciens* MTCC 431; *Bacillus cereus*, two fungal cultures *Aspergillus niger* and *Candida glabrata* procured from microbial type cultural collection (MTCC), were used as test organisms. By agar well diffusion procedure, antimicrobial activity was resolved. The disc was stained in 15 micro liter of extract separately. Agar well diffusion method was used at different concentration, MIC of selected methanilol extract was resolved. Against *Bacillus cereus*, the methanolic extract of different parts of *A.nilotica* displayed good against bacterial assay. The maximum zone of reticence was calculated in leaf extract against *E.coli*, (15.30 mm) followed by bark extract against *C. glabarata* (15.00 mm). Against *Cereus* and *E. coli*, seed extract also displayed anti bacterial activity against *C. glabarata*, the antifungal activity was noticed. Minimum inhibitory concentration (MIC) of methanol extract of leaf against *E. coli* ABCDE (Different concentration of extract in well (A=5, B=10, C=15, E=25)).

Presence of Glycosides, flavonoids, alkaloids, saponins tannin and resin were observed in methanolic extract. Against test organisms designed, these might be responsible for antimicrobial activity. This plant was utilized for the treatment of Diarrhoea, Skin infections, other infectious diseases and skin diseases (Diame, 2010; Umair et al., 2017; Baana et al., 2018; Umair et al., 2022).

Conclusion

The study has shown that *Vachellia nilotica* is a rich source of phytochemicals and nutrients. Medicinal plants represent a vital source of food in the lives of poor populations, they act as a source of fuel wood, construction material and other basic needs. Medicinal plants are also a mean of drugs and therapeutic agents. Organic compounds found in these plants exhibit a

physiological and biological action on the body of humans. The bioactive substances found in *Vachellia nilotica*. after respective tests were Carbohydrates, Steroids, Terpenoids, flavonoids, phenols, amino acids, proteins. It may also be used for the formation of organic amendment agents as it has flavonoids and alkaloids which may be used as antifungal agent for crop protection as well. Hence from the present study, it is concluded that *Vachellia nilotica* can be used as constituents in the formation of several herbal as well as synthetic drugs.

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