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Vachellia nilotica, a potential plant source candidate to reduce the oxidative stress and to kill the bacteria

[1] CS Padmasree, [2] Nahid Akhtar, [*3] Navneet Kumar

[1, 3] Department of Biochemistry, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

[2] Department of Molecular Biology and Genetics, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

[3] Department of Biochemistry, School of Life Sciences, Central University of Rajasthan, Bandar Sindri, Ajmer (District), Kishangarh, Rajasthan, India

1 & 2: Equal Contributors

Abstract
Oxygen is life supporting gas and notorious too. It is used by our body for various purposes including for formation of free radicals for different reasons. But, if these free radicals are produced in excess, in spite of free radicals scavenging machinery in our body, they have the destructive property of membrane, DNA, RNA, and proteins. The plants are found to be useful for adding the scavenging ability in our body because of the presence of valuable phytochemicals present in them. Keeping this under consideration, *Vachellia nilotica* was here studied for determination of phytochemicals present in it and then for evaluation of the antioxidant activity. Not only this, but phytochemicals present in plants can show antimicrobial activity also. Thus, the extract was used to determine the antimicrobial activity in this study. Qualitative study revealed the presence of various phytochemicals such as tannins, flavonoids, terpenoids, saponins, and phenols whereas quantitative study showed the presence of polyphenolic compounds in ample amount. It also showed the significant antioxidant and antimicrobial activity. Thus, the present study demonstrated the potential of *V. nilotica* to reduce the oxidative stress and to kill the bacteria including gram-positive as well as gram-negative.

Keywords: *vachellia nilotica*, antioxidant, free radicals scavenging, antimicrobial, phytochemicals

1. Introduction
The oxidation of foods by oxygen to generate energy is significant for the sustenance of life, but the oxygen also gives rise to oxidative stress which is harmful to us. Oxidative stress arises when different free radicals formed during a variety of metabolic processes and exposure to stress, pollutants, and smoke are not efficiently scavenged. The oxidative stress causes oxidation of DNA, lipids, and proteins which is deleterious to the cells and is also involved in the progression of several diseases such as atherosclerosis, aging, cardiovascular diseases, neurodegenerative diseases and other inflammatory diseases [1,2,3]. Another emerging threat is of antibiotic resistance in bacteria which has caused havoc world over. Diseases caused by bacterial and fungal infections have troubled people for ages. The currently available treatment of such diseases is the use of antibiotics, but due to our negligence and unnecessary use of antibiotics, bacteria have evolved and become resistant to antibiotics. In this regard, plants can be a good source of potential antimicrobial and antioxidant compounds. There are references to plants and herbs being used by monks to cure various diseases in several classical Indian and non-Indian literature. They are still being used by tribal and are quintessential element of Ayurveda, Chinese herbal medicine, Unani medicine and Siddha medicine. Even in the modern world, in remote places of the world such as Africa around 80% of the people rely on herbs and plants for the treatment of diseases [4]. In the developed nations Europe and North America also the use of natural plant-based products is increasing due to the reported side effects of the conventional drugs [5]. Previous studies on several other plants such as *Artemisia indica*, *Leucas aspera*, *Phyllanthus niruri* have shown that plants are rich in antioxidants and antimicrobial compounds [6,7,8]. Considering this, the analysis of phytochemical constituents, antimicrobial activity and antioxidant property of the plant *Vachellia nilotica* was studied.

V. nilotica is a gum oozing tree belonging to Fabaceae family with flowers native to Egypt which has now been naturalized to different parts of the world such as India, Pakistan, Australia, Africa and Middle East [9,10]. The tree is commonly called "Arabic gum tree in English, "Babool" in Hindi and "Arul" in Malayalam. The plant is widely used in Unani medicine as astringent, laxative, and tonic for eyes and nerves [11]. The leaves are used to cure a sore throat, wound ulcers and eye diseases [11]. The gum is used as an aphrodisiac, expectorant and antipyretic substance [11]. The plant has also shown anti-malarial, anti-cancer and anti-fungal activity [12, 13, 14].

2. Material and Method

Chemicals required

2, 2 diphenyl 1-picrylhydrazyl (DPPH), Aluminum chloride ($AlCl_3$), Ammonium molybdate, Ascorbic acid, Ferric chloride ($FeCl_3$), Folin Coicateau reagent, Gallic acid, Methanol, Phosphate buffer, Potassium acetate, Potassium ferricyanide, Quercetin, Sodium carbonate (Na_2CO_3), Sodium dihydrogen phosphate (NaH_2PO_4), Sulfuric acid (H_2SO_4), Trichloroacetic acid (TCA).

Sample Collection and Extract Preparation

Leaves of *Vachellia nilotica* were collected from Kollam district in Kerala during the month of December. The collected leaves were washed thoroughly by removing mud and other dust particles. Then these leaves were dried in the shade at room temperature and powdered by grinding. 30gm of dried leaves powder was measured and extracted using Soxhlet apparatus with methanol as solvent. The extract was concentrated to dryness under controlled temperature (40-50ºC) and then preserved in the refrigerator.

Microbes Used

The bacterial strain of *Lactobacillus acidophilus, Pseudomonas species, Staphylococcus aureus, Escherichia coli* were used for the antimicrobial activity.

Phytochemical screening

The screening of phytochemicals present in *V. nilotica* was performed by following the protocol in Harbone[15] for the qualitative detection of terpenoids, flavonoids, saponins, tannin, and phenols were performed.

Total flavonoid content

The aluminum chloride ($AlCl_3$) method is utilized for the determination of total flavonoid content with the utilization of spectrophotometer [16]. Plant extract (0.2ml of 100µg/ml) was prepared in 0.6ml methanol. Aluminum chloride (40µl, 10 %), Potassium acetate (40µl, 1M) and distilled water (1120µl) were added in the given order and incubated for 30 minutes at room temperature. Absorbance was taken at 420 nm using ELICO SL 210 UV VIS spectrophotometer. Quercetin was used as standard, and all the experiments were performed in triplicates and mean value was taken.

Total Phenol Content

Folin Coilcateau method was used to measure the total phenolic content of *V. nilotica* [8]. Plant extract (40µl) was mixed with 2 % Na_2CO_3 (2ml), 10 % Folin Coicateau reagent (2.5 ml) and vortexed. The reaction mixture was incubated for 15 minutes at 45^0C in a water bath. Absorbance was taken at 765 nm. All the tests were done in triplicates and mean values were considered. Gallic acid was used as the standard compound.

Total Antioxidant Activity Assay

The total antioxidant activity of the methanolic plant extract was assessed by the phosphomolybdenum assay as indicated by the method by Prieto *et al.* [17]. 0.3mL of concentrate was mixed with 3ml of reagent arrangement (0.6M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate). The tubes containing the reaction mixture were incubated at 95ºC for 90 min. Then, the absorbance of the mixture was measured at 695nm with a UV-VIS spectrophotometer (UVmini-1240). Ascorbic acid was used as a standard to construct a standard graph.

Reducing Power Assay

The reducing power of every extract was decided by the method described by Oyaizu *et al.* [18]. Volumes of 2.5mL of various concentrations of the extract (1000, 500, 250, 125, 62.5 and 31.25 µg/mL) were blended with 2.5 mL phosphate buffer solution (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferric cyanide in test tubes. The mixture was set in a water bath at 50ºC, for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture and vortexed. A volume of 2.5mL of this mixture was then added to 2.5mL of distilled water and 0.5mL of $FeCl_3$ (0.1%), and then the absorbance of the mixture was measured at 700nm utilizing a UV-VIS spectrophotometer. Ascorbic acid was utilized as a standard.

DPPH Scavenging Activity

The in vitro antioxidant property of the 90% methanolic extract of *V. nilotica* leaves was determined by 2, 2 diphenyl 1-picrylhydrazyl (DPPH) free radical scavenging activity according to Liyana-Pathiana and Shahidi [19]. 0.135mM DPPH was dissolved in 100% methanol. Plant extract concentration 0, 5, 10, 15, 20, 25, 30, 35 and 40µg/ml was prepared. 1ml of 0.135mM DPPH and 1ml of plant extract of different concentrations were mixed in test tubes and incubated for 30 minutes at room temperature in the dark. Then, the absorbance was measured at 517nm using ELICO SL 210 UV VIS spectrophotometer. Ascorbic acid was used as a standard. The DPPH scavenging activity was calculated as:

$$\%\text{DPPH scavenging activity} = \{(Abs_{control} - Abs_{sample})/Abs_{control}\} \times 100$$

Where,

$Abs_{control}$ = Absorbance of methanol + DPPH

Abs_{sample} = Absorbance of sample (plant extract/ Ascorbic acid) + DPPH

Antibacterial Assay

The antibacterial property of *V. nilotica* was evaluated using agar gel diffusion method [7]. Nutrient agar media was autoclaved and after cooling was poured onto Petri dishes. Bacterial culture (100 µl) was spread on the plates then the plates were permitted to dry for 30 min, and 6 mm diameter wells were made on the agar plates using sterile cork borer. To diffuse the compound into the agar, the plates were permitted to remain at room temperature for 1h and then the plates were incubated at 37°C for 24h and inspected for development of bacterial inhibition zone. The inhibition zone diameter (IZD) was measured in millimeter. Vancomycin was taken as positive control for both the gram-positive bacteria and the gram-negative bacteria. 100µl of plant extract (50µg/ml) was filled in each well.

3. Results

Phytochemical screening

Generation of free radical or reactive oxygen species causes oxidative stress in our body and causes DNA damage, lipid peroxidation which accelerate chronic disorders like cancer, arthritis, cataract and other neurodegenerative diseases [1, 2, 3]. The phytochemicals present in various plant parts possess significant antioxidant activity which helps to scavenge the free radicals to prevent the oxidative stress. In the present study, the presence of different phytochemicals such as tannin, saponin, phenolics, alkaloids, terpenoid was qualitatively estimated by standard methods. The extract of *V. nilotica* showed a positive result for tannins, saponins, terpenoids, phenols, and flavonoids (Table 1). These phytochemicals have shown to have antioxidant and antimicrobial activity.

Polyphenolic compounds

Considering the qualitative determination of phytochemicals, phenols and flavonoids were quantitatively determined for the better understanding of the extent of antioxidant and antimicrobial activity. Most antioxidants segregated from medicinal plants are polyphenols which demonstrate antibacterial and antioxidant activity [20]. From experimentation of total phenolic content, it was found that 1 mg of *V. nilotica* contains 178.57 µg of the equivalent of gallic acid. Flavonoids are plant's secondary metabolite which gives yellow, red or blue pigmentation to plants and provide antioxidant activity to plants. By the help of standard graph of quercetin, it was found that 1 mg of *V. nilotica* contains 444.16 µg equivalent of quercetin.

Antioxidant status

After testing total phenolic content and total flavonoid content, we measured their expected effects on antioxidant status through DPPH free radical scavenging assay, total antioxidant, and total reducing power. Renu *et al.* on the antioxidant activity of plants mentioned that the plant with highest phenolic and flavonoid content demonstrate the highest antioxidant and reducing power [21]. For DPPH scavenging activity assay, the IC50 value of *V. nilotica* was found to be 35.58µg/ml compared to the IC50 value of reference compound ascorbic acid with IC50 value 7.38µg/ml. The reducing power of the methanolic extract was found to be equivalent to 52.04µg of gallic acid per 1mg of the plant extract. The total antioxidant activity of the methanolic extract was found to be equivalent to be 92µg of ascorbic acid per milligram of plant extract. The high reducing and antioxidant power could be correlated with the presence of high amount of polyphenolic compounds.

Table 1: Qualitative phytochemical screening for *Vachellia nilotica* plant extract

Serial Number	Phytochemicals	Method	Observation	*Vachellia nilotica*
1	Saponins	Emulsion formation test	Formation of emulsion	Positive
2	Alkaloids	Mayer's reagent test	No characteristic changes	Negative
3	Phenols	Ferric chloride test	Blue color appeared	Positive
4	Terpenoids	Sulphuric acid test	Reddish brown color	Positive
5	Flavonoids	Ammonia test	Yellow color disappeared from standing	Positive
6	Tannins	Ferric chloride test	Blue-black color	Positive

Antimicrobial activity

The presence of various phytochemicals such as polyphenolic compounds in plant extracts may account for the antimicrobial activity. The methanol extract of *V. nilotica* leaves was all to inhibit the growth of all the bacteria under study. Vancomycin was used as a standard antibiotic. The zone of inhibition formed by Vancomycin was 22 mm, 20 mm, 17 mm and 19 mm for *Pseudomonas species*, *Lactobacillus acidophilus*, *Staphylococcus aureus* and *Escherichia coli*, respectively. The extract had the zone of inhibition with diameter 7mm, 9mm, 10mm, and 8mm, respectively for Pseudomonas species, *L. acidophilus*, *S. aureus* and *E. coli*. The plant extract had maximum antimicrobial activity against gram-positive bacteria *S. aureus*.

Conclusion

Vachellia nilotica is a tree whose leaves, gum, root, and bark are used in Unani medicine and ethnomedicine world over as astringent, gargle, laxative, a tonic for eyes and aphrodisiac. Considering this, the phytochemical constituents, antioxidant property and bactericidal activity of this plant were evaluated. The methanol extract of the plant showed the presence of tannin, saponins, terpenoids, phenols, and flavonoids. Similarly, phenols and flavonoids were also determined quantitatively. The plant showed a good antioxidant activity and was able to inhibit the growth of both gram-negative as well as gram-positive bacteria. This study signifies the use of the plant in traditional medicine and also shows that the plant is rich in antioxidants and antimicrobial compounds.

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