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Study on methanolic extract of *Ageratum conyzoides* for its ability to act as an antioxidant and to suppress the microbial growth

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Abstract

Oxygen is a life-supporting gas for us. One of the purpose of it is to make radicals in the body in a controlled fashion which are used by the body. But, if their production is in excess, they may lead to various ailments in the body like cancer, cardiovascular problems, Parkinson, inflammation, etc. To avoid this, these radicals are scavenged by the body antioxidant system. This scavenging can be further supported by the plant products which are rich in phytochemicals and are having role in scavenging. Present study is dealing to determine the antioxidant potentials of the methanolic extract of the *Ageratum conyzoides* through DPPH radical scavenging assay, total antioxidant status and total reducing power. Other than that, microbes are a threat to us and this danger is further aggravated by the development of the resistance against antibiotics in them. The present study is also determining the microbiocidal ability of the plant extract. We further assessed the total phenolic and flavonoid content which could be one of the factors for showing antioxidant and antimicrobial potentials. Our study showed that *A. conyzoides* was having significant antioxidant and antimicrobial potentials. It was also having ample amount of the phenolics and flavonoids. These potentials are required to further establish for assigning the future uses.

Keywords: *Ageratum conyzoides*, antioxidant, antimicrobial, plant extract

Introduction

Oxygen is substantial for the sustenance of life. However, free radicals generated from oxygen in our body during various metabolic processes and exposure to stress, smoke and pollutants have deleterious effects on our health [1]. Free radicals, which are highly unstable molecules with an unpaired electron, help our body to defend the invasion of bacteria and viruses [2]. But, when the free radicals are available in excess they initiate oxidative stress that oxidizes vital biomolecules such as DNA, proteins, and lipids thus damaging the cell and disrupting the homeostasis [3, 4]. Oxidative stress has been associated with diseases like cancer, heart disease, atherosclerosis, Parkinson, Alzheimer and various other inflammatory diseases [5, 6].

To annul the damages of oxidative stress, our body has developed an antioxidant system which entails enzymes such as glutathione peroxidase, catalase, superoxide dismutase to quench the free radicals thus preventing the oxidation of biomolecules and preventing the cellular damage [7]. Apart from the natural antioxidants, plants, fruits, and herbs also supplement us with antioxidants exogenously [8]. Previous studies have shown the abundant presence of antioxidants in traditionally used plants and herbs such as *Phyllanthus niruri* and *Leucas aspera* to name a few [9, 10]. These plants have been able to exhibit the antioxidant activity due to the presence of phytochemicals such as phenols, terpenoids, and flavonoids [11, 12].

The conventional drugs used to cure diseases caused by bacterial infections have mild to severe side effects, and the development of resistance to standard antibiotics have further exacerbated the plight thus implying that the development of novel and efficient antimicrobial compounds is exigent [13]. In this regard, plants have also shown potential to be the source of phytochemicals with antimicrobial activity [9, 10].

The use of plants in traditional medicine and Ayurveda to cure grave ailments bolsters the fact that plants are rich in antioxidants and antimicrobial phytochemicals. Considering this the antimicrobial activity and antioxidant property of *Ageratum conyzoides* was evaluated. *A. conyzoides* is a member of the Asteraceae family and is commonly found in Africa, South East Asia, India, Nepal, Australia and South America [14]. This herb is indigenous to America, and it's been introduced and naturalized in India. Commonly it is called "billy goat weed" and as "otogo," and "ufu opioko" in Nigeria [14, 15].

The essential oil of *A. conyzoides* is rich in sequesterines, terpenes, alkaloids, coumarin, ageratochromene, kaempferol [14, 16]. In previous studies, the plant has shown antidiabetic activity, wound healing activity, anti-inflammatory activity and insecticidal property [14, 17-19]. Likewise, *A. conyzoides* has been known for its medicinal properties since ancient times and is utilized for the treatment of various ailments such as headache, pneumonia, asthma, inflammation, burns and wounds, stomach ailments, leprosy, diabetes, gynaecological diseases and various skin diseases [17, 20]. The concentrate or infusion of the plant is used in dysentery, diarrhea, flatulence, intestinal colic, rheumatism and fever [21].

As it is shown that the plant has therapeutic capabilities, the antioxidant activity, phytochemical analysis, and antimicrobial activity were assessed. The antioxidant activity was evaluated by DPPH radical scavenging ability, ferric reducing assay, and total antioxidant assay. Phytochemical analysis was done to assess the phenol and flavonoids quantitatively, and antimicrobial activity was evaluated by agar gel diffusion method against both pathogenic and non-pathogenic bacteria.

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

Leaves of *Ageratum conyzoides* were collected from Darrang district of Assam in India during the last week of December 2016. The leaves were washed thoroughly to get rid of soil and dust and then dried. The dried leaves were crushed and milled to a fine powder with a mixer grinder.

Preparation of the extract

Leaf powder was measured, and the extract was prepared using Soxhlet apparatus taking 90% methanol as solvent. The apparatus was run successively for 48 hours, and then the extract was concentrated to dryness using rota-vapor maintaining a temperature of 55°C . The samples were stored in the refrigerator for further experimentation.

Quantitative Phytochemical Analysis and Evaluation of Antioxidant Activity

Total Flavonoid Content

The flavonoid content of *A. conyzoides* was determined quantitatively according to Aluminum Chloride reducing method [22]. Plant extract (0.2 ml) of *A. conyzoides* having concentration $400\mu\text{g/ml}$ in methanol was taken. Aluminium chloride ($40\mu\text{l}$, 10%), potassium acetate ($40\mu\text{l}$, 1 M) and distilled water ($1120\mu\text{l}$) 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. All the experiments were performed in triplicates and mean value was considered. Quercetin was used as a standard compound.

Total Phenol Content

Folin Coicateau method was used to measure the total

phenolic content of *A. conyzoides* [23]. Plant extract (0.5 ml of 1mg/ml) was mixed with 2% Na_2CO_3 (2ml), and 10% Folin Coicateau reagent (2.5 ml) and vortexed. The reaction mixture was incubated for 15 minutes at 45°C in a water bath. Absorbance was taken at 765 nm. All the tests were done in triplicates and mean values were taken. Gallic acid was used as the standard compound.

DPPH Scavenging Activity

The in vitro antioxidant property of the 90% methanolic extract of leaves of *A. conyzoides* was evaluated by 2,2 diphenyl 1-picrylhydrazyl (DPPH) free radical scavenging activity according to Liyana-Pathiana and Shahidi [24]. 0.135mM DPPH was prepared in 100% methanol. Plant extract ranging from concentration 20-400 μg (*A. conyzoides*) was prepared. 1ml of 0.135mM DPPH and 1ml of plant extract of different concentrations were mixed in test tubes and incubated in dark for 30 minutes at room temperature. After incubation the absorbance of the mixture was taken at 517nm using ELICO SL 210 UV VIS spectrophotometer. Ascorbic acid was used as standard and all the tests were done in triplicates and mean value was taken. The DPPH scavenging activity was calculated as:

$$\% \text{DPPH scavenging activity} = \left\{ \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right\} \times 100$$

Where,

$\text{Abs}_{\text{control}}$ = Absorbance of methanol + DPPH

$\text{Abs}_{\text{sample}}$ = Absorbance of sample (plant extract/ Ascorbic acid) + DPPH

Ferric reducing power assay

The reducing power of methanolic extract of *A. conyzoides* was evaluated by following the method of Oyaizu *et al.* [25]. Plant extract having concentration from 100-200 μg was prepared in distilled water. Plant extract (1ml) of different concentrations was mixed with phosphate buffer (1.5ml, pH 6.6, 0.2M) and 1% potassium ferricyanide (1.5ml) and incubated in water bath for 20 minutes at 50°C , followed by addition of trichloroacetic acid (10%, 1.5ml) and centrifuged at 4000 rpm using REMI R-8C laboratory centrifuge for 20 minutes. Supernatant (1.5ml) was collected and mixed with distilled water (1.5ml) and ferric chloride (0.1%, 0.3 ml). The mixture was vortexed and absorbance was measured using ELICO SL 210 UV VIS spectrophotometer at 700nm. The experiments were done in triplicates and mean value was taken. Gallic acid was used as a standard reference compound for the study.

Total Antioxidant Activity

The overall antioxidant activity of *A. conyzoides* was evaluated in vitro according to the phosphor-molybdenum method by following the procedure of Prieto *et al.* [26]. Plant extract of different concentrations in ethanol (0.1 ml) was mixed with 1.9 ml reagent (28mM NaH_2PO_4 , 4mM Ammonium molybdate, and 0.6M H_2SO_4) and incubated in water bath at 95°C for 90 minutes. After incubation absorbance was taken at 695 nm using ELICO SL 210 UV VIS spectrophotometer. Ascorbic acid was used as standard, and all tests were done in triplicates. Mean values were taken.

Antibacterial Assay

Five bacterial strains namely *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella enterica* and

Pseudomonas putida were used for the antibacterial assay which were provided by Department of Microbiology, Lovely Professional University, Phagwara, India. The glycerol stocks of these bacterial strains were revived in nutrient broth by incubating at 37°C for 24 hours.

The antibacterial properties of these plants were assessed using agar gel diffusion method. Nutrient agar media was autoclaved and after cooling was poured onto Petri dishes. Bacterial culture (100µl) was spread on the plates after solidification of the plates. Wells were made on the solidified media, and plant extracts were filled in the wells. Plant extract used had the concentration of 20mg/ml, 50mg/ml and 100mg/ml. Tetracycline (30 mcg) and DMSO were used as a control.

Result and Discussion

Herbal remedies are used for curing diseases since antiquity world over. They are an integral part of Siddha medicine, Ayurveda, and Unani medicine. These plants are rich in phytochemicals with antimicrobial activity and antioxidant activity [9, 10]. These antioxidant phytochemicals counterbalance the free radicals thus preventing the oxidative stress which is responsible for several diseases. The phytochemicals such as phenolics are also able to inhibit the bacterial growth thus acting as a source of potential antimicrobial compounds [27]. Thus, the plant-based compound can be a cheap and efficient alternative to convert drugs which have side effects and also solve the ever-expanding problem of antibiotic resistance. Considering this the methanol extract of *Ageratum conyzoides* was studied for its antioxidant and antimicrobial activity.

Polyphenolic Compounds

The total phenolic content of methanolic extract of *A. conyzoides* was found to be 8.52µg equivalents of gallic acid per milligram of extract. The presence of phytochemicals especially phenolic compounds such as phenolic acids, flavonoids, tannins, curcuminoids, quinines, etc. are important to scavenge the free radicals [27]. The total flavonoid content of *A. conyzoides* was 9.575µg equivalents of quercetin per milligram of crude plant extract. The presence of phenolic compounds and flavonoids provided the evidence that the plant may have antimicrobial and antioxidant activity. Thus, these properties were evaluated experimentally.

Antioxidant Status

DPPH radical scavenging ability assay was performed to determine the antioxidant activity of the plant extract. The results showed that the antioxidant activity of *A. conyzoides* increased with increase in the concentration of plant extract. The methanol extract of *A. conyzoides* showed the IC50 value of 213.57µg/ml against the IC50 value of reference compound ascorbic acid with IC50 value 6.82µg/ml. As the extract was in crude form, it showed less DPPH scavenging activity than the pure antioxidant compound ascorbic acid. The reducing power of the extract of *A. conyzoides* was found to be equivalent to be 41µg of gallic acid per milligram of extract. The total antioxidant activity of *A. conyzoides* methanol extract was determined to be equivalent to 316µg of ascorbic acid per milligram of extract. The antioxidant activity of the extract was probably due to the presence of flavonoids and phenolic acids. Not only this, other compounds present in the plant extract may have further contributed significantly to the antioxidant activity of the plant extract.

Antimicrobial Activity

The antimicrobial activity of plant extract was determined against both gram-positive and gram-negative bacteria, some of which were pathogenic and some were non - pathogenic. The crude extract was able to inhibit the growth of all bacteria under study. The diameter of the zone of inhibition formed by *A. conyzoides* (100mg/ml) for *E. coli*, *S. aureus*, *P. putida*, *S. enterica* and *S. pyogenes* were 14mm, 18mm, 13mm, 12mm and 10 mm, respectively. Tetracycline was used as a standard. The plant extract showed highest antibacterial property against *S. aureus*, a gram-positive bacteria.

Conclusion

Ageratum conyzoides is traditionally important plant that has been used since ages as traditional remedies for the treatment of a wide range of health disorders. In the present study, the plant was proved to possess good antioxidant and antibacterial potentials. This study also corroborates the use of the plant as medicine in ethnomedicine to cure several infections. It inhibited the growth of different gram-positive and gram-negative bacteria, which are often potent pathogen to human health. These promising properties may be due to the presence of various phytochemicals such as phenolic and flavonoids. Further studies need to be done to determine the other pharmacological properties of the plant, and its prospective use in various industries.

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