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Determination of Falconoid Content in Parthenium Hysterophorus and Comparative Study of Antibacterial Activities

Shahnawaz Khan, Nikunaj Bhardwaj
Department of Biotechnology, Noida International University, Greater Noida (UP), India
Corresponding Author: Shahnawaz Khan

Abstract: There is a need for human society to discover new antimicrobial compounds having diverse chemical structures and innovative mechanisms of action for novel and reemerging infectious diseases. Therefore, researchers are progressively turning their consideration to traditional medicine, looking for new leads to develop improved drugs against microbial infections. Plants yield a diverse variety of bioactive molecules, making them rich source of different types of medicines. Parthenium hysterophorus is a weed plant reported to be used as remedy for a number of diseases. Present work shows the activities of Parthenium hysterophorus L. stem, flower, and root. Samples were sequentially extracted with many solvents. Flower extracts exhibited presence of higher amount of flavonoids followed by stem, and root. Stem and root fractions showed noticeable antioxidant capacity in phosphomolybdate assay. The study open antibacterial potential in stem, flower, and root parts of Parthenium hysterophorus L.

Keywords: Antimicrobial, Parthenium hysterophorus L., Pathogens, Well diffusion method.

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I. INTRODUCTION

The life of each organism present in the world is based on the green vegetation which it lives around. Every organism in this universe has a specified role to play which determine its role in the ecosystem. Many play a conservative role, among those plants are being the prime base as they are sustaining our environment. Even several centuries before the invention of modernized equipment and drugs, plants provided cures for many severe medical illnesses. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. [1] In the recent years we were hugely depended on the commercial and synthetic drugs which have resulted in the adverse side effects, resistance among several pathogenic organisms and much more. This scenario pushed us to go back to our mother of all producers, the plants to look for effective medicine of lesser or no side effect. *Parthenium hysterophorus* L. from the family of Asteraceae, popularly known as Congress weed, Carrot weed, Star weed, Fever few, White top, Chatak Chandani, Bitter weed. The ability of its seeds to germinate in any season of the year, makes it a constantly flourishing component of the vegetation.[2]

Parthenium is also reported as a promising remedy against hepatic amoebiasis.[3] South American Indians use the decoction of roots to cure ambiotic dysentry, [4] whereas parthenin, a toxin of Parthenium, is found pharmacologically active against neuralgia and certain types of rheumatism. It is applied externally on skin disorders and decoction of the plant is often taken internally as a remedy for a wide variety of ailments.[5,6]

In this study, we had evaluated the phytochemical screening and antimicrobial activity of *Parthenium hysterophorus* L. against various clinical isolates of pathogenic bacteria and fungi.

Oxidative stress is a pathophysiological condition arises because of overproduction of reactive free radicals. These free radicals overproduce by the imbalance in redox couples during metabolism. The products of this imbalance are molecules that are enriched in one or more oxygen atoms known as the reactive oxygen species that are generally considered to be markers of oxidative stress [1–3]. Cells possess several antioxidant enzymes and molecules to combat reactive oxygen species induced damage [4]. Potential of antioxidant as an anticancer agent depends on its competence as an oxygen radical inhibitor. It has been found that the percentage of deaths from cancer is slightly higher in males than in females [5]. Plants Phytoconstituents have been a source of medicine since time ancient. Active phytochemicals can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, and so forth. Currently, over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources [6, 7]. Flavonoids are polyphenolic compounds having antioxidative, antiviral, and anticancer activities. They are produced by plants as well as by genetically

modified microorganisms [8]. Infectious diseases are responsible for large scale morbidity and mortality worldwide. For example *Salmonella typhi* causes typhoid fever and is exclusively adapted to infection of the human host. It has been observed that approximately 600,000 deaths annually occur worldwide because of typhoid fever [9]. *Streptococcus mutans* is associated with pyogenic and other infections in various sites including mouth, heart, and central nervous system [10]. *Proteus vulgaris* is associated with urinary tract infections [11]. Treatment of infectious diseases with currently available drugs is associated with various side effects in addition to emergence of drug resistant bacterial strains [12]. Hence, it is imperative to discover novel antibacterial agents from natural sources that may have lesser side effects. There are many studies showing antimicrobial activities of the extracts prepared from different type of plants [13–15]. Picman and Picman mentioned in 1984 that *Parthenium hysterophorus* L. is an aggressive weed native to Southern North America, Central America, the West Indies and Central South America. Tefera in 2002 discovered its allelopathic effect and drastic retardation of the growth of many species. Parthenium hysterophorus L. is a well-known weed that is a serious problem in agriculture practices. Lalitha et al., 2012 said that Parthenium hysterophorus produces a toxin called parthenin.

Parthenium hysterophorus L., of the family Asteraceae (tribe: Heliantheae), is a straight and greatly branched annual or ephemeral herb, well-known for its notorious role as environmental, medical, and agricultural hazardous activities. It is believed to have been introduced into India and Australia from North America and in the last few years the weed has emerged as the seventh most devastating weed in Africa, Asia, and Australia. All parts of the weed are reported to be used as bitter tonic, febrifuge, emmenagogue, antidysenteric, and so forth. It has been found that *Parthenium hysterophorus* pharmacologically active as analgesic in muscular rheumatism and as vermifuge and therapeutic for neuralgia [16].

II. MATERIALS AND METHODS

The *Parthenium hysterophorus* stem, flower, and root were shade-dried, crushed, and ground into fine powder with mortar and pestle. Powdered material was sequentially extracted with different solvents and water in Soxhlet apparatus as described earlier [17, 18]. After that the extracts were centrifuged, filtered, and lyophilized. The dried residues were dissolved in DMSO for determination of antibacterial, antioxidant, and anticancer activities. *Streptococcus mutans* and *Salmonella typhi* were procured from the Noida Testing Laboratory, Noida, India. The bacterial culture was maintained at 4◦C on nutrient agar slants.

Antimicrobial Activity

Antimicrobial activity of plant extracts was determined using Kirby-Bauer disc diffusion method [19]. The inoculum suspension of bacterial strains was swabbed on the entire surface of Mueller-Hinton agar. Sterile 6 mm diameter paper discs saturated with 20 L of extracts prepared in DMSO (2 mg extract/disc) were aseptically placed on the upper layer of the inoculated Mueller-Hinton agar surfaces and plates were incubated at 37◦C for 18 hours. Antibacterial activity was determined by measuring diameter of the zone of inhibition surrounding discs. Standard antibiotic discs meropenem and piperacillin tazobactam were used as positive controls. Discs containing 20 L DMSO was used as a negative control.

Total Flavonoid Content

For the determination of flavonoids in various extract fractions of *Parthenium hysterophorus* stem, flower, and root we use aluminum chloride colorimetric method [20] modified by A. Mishra, S. Kumar, A. Bhargava, B. Sharma and A. K. Pandey, 2011 [21]. 0.2 mL of extract in pure DMSO was separately mixed with methanol (1.8 mL), 10% aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), and distilled water (2.8 mL). Tubes were incubated at room temperature for 30 min. Now absorbance of the reaction mixture was measured at the 415 nm. After that the calibration curve was prepared with the quercetin solution. Changed volumes containing 20–200 g quercetin were taken in diverse tubes and volume was raised to 1.8 mL with methanol monitored by addition of 0.2 mL DMSO. Remaining procedure was followed similarly as described above.

The phosphomolybdate method using propyl gallate as standard [22] with some modification done by the A. Mishra, S. Kumar, A. Bhargava, B. Sharma and A. K. Pandey, 2011 [21] was used to determine the total antioxidant capacity of the extract fractions. To 0.05 mL (100 g) of the extract solution prepared in DMSO, 0.25 mL methanol was added followed by the addition of 3 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95∘C for 90 min leading to development of green colour. After that samples had cooled to room temperature. The absorbance was measured at 695 nm.

The antioxidant activities of P. hysterophorus extracts were investigated according to the method described by H. J. D. Dorman et al. 2000 [23]. Two gram of agar was completely dissolved in 100 mL hot water and the solution was allowed to cool to 50∘C followed by addition of 4 mL linoleic acid and 20 mL of -

carotene. The agar was poured into Petri dishes and allowed to set for 30 min. 4 mm Wells were punched into the agar of each Petri dish using a sterile cork borer. Plant extracts prepared in DMSO was added to each well. The plates were incubated overnight at the 45∘C until the background colour had bleached.

LC-MS analyses of *P. hysterophorus* extracts were performed on an Agilent Technologies 1200 series UPLC with 6540 series electro-spray ionization, MS mode. Samples were dissolved in methanol and water. The separation was achieved using Agilent Technologies, reversed-phase column held at 45◦C.

Zone of inhibition values are reported as average of three replicates. The extract contents present in the discs were 2 mg/disc. The values are represented as g quercetin equivalent per milligram of sample (g QE/mg). The results are expressed as mean ±SEM (= 3). The fragmentor voltage was 135 V and injection volume for all samples was 5 L. The binary mobile phase consisted of water (solvent A) and acetonitrile (solvent B). Gradient elution was performed using the following solvent. Percentage of solvent B was 10, 10, 50, 90, 90, 50, and 10 % at 0.01, 2, 12, 22, 35, 45, and 50 min, respectively, at a flow rate of 0.7 mL/min.

III. RESULTS

Antibacterial Activities

The antibacterial activities of the extracts derived from stem, flower, and root of *Parthenium hysterophorus* were evaluated against Gram positive *Streptococcus mutans* and Gram negative and *Salmonella typhi* bacterial strains. Among *Parthenium hysterophorus* stem extracts nonpolar fractions showed moderate antibacterial activity against *S. mutans* while ethanol extract showed considerable antibacterial activity. *S. typhi* exhibited resistance to all the stem extracts. Hexane, benzene, ethyl acetate and aqueous extracts of *Parthenium hysterophorus* flower were active against *s. mutans.* Flower hexane extract accounted for noticeable inhibitory activity against *S. mutans.* Similarly, hexane, chloroform, Ethyl acetate, and aqueous fractions of *Parthenium hysterophorus* root extracts showed antibacterial activity against *S. mutans.* However and *S. typhi* showed resistance against flower and root extracts.

Among *P. hysterophorus* stem extracts nonpolar fractions showed moderate antibacterial activity (zone of inhibition, 11–18 mm) against *S. mutans* while Ethyl alcohol extract showed considerable antibacterial activity (zone of inhibition, 18 mm). Generally *S. typhi* exhibited resistance to all the stem extracts. Hexane, Benzeene, Ethyl Acetate, and Aqueous extracts of *P. hysterophorus* flower were active against *S. mutans*. Flower Hexane extract accounted for noticeable inhibitory activity (zone of inhibition, 17 mm) against *S. mutans*. Similarly, Hexane, chloroform, Ethyl acetate, and Aqueous fractions of *P. hysterophorus* root extracts showed antibacterial activity (zone of inhibition, 10–17 mm) against *S. mutans.* However, *S. typhi* exhibited complete resistance against flower and root extracts.

Total Flavonoid Content

It has been observed that amount of flavonoid contents varies in all the extracts of weed. Comparatively higher amount of flavonoid content was found in *Parthenium hysterophorus* stem ethanol, and flower chloroform, ethyl acetate, and acetone extracts. *Parthenium hysterophorus* flower fractions exhibited higher flavonoid content. Comparatively higher amount of flavonoid content was found in *P. hysterophorus* stem Ethyl Alcohol, and flower Chloroform, Ethyl acetate, and Acetone extracts. *P. hysterophorus* flower fractions exhibited higher flavonoid content.

The *Parthenium hysterophorus* weed extracts showed numerous degrees of antioxidant capacity. Among all the test extracts of *Parthenium hysterophorus* stem benzene fraction showed maximum antioxidant capacity. Antioxidant activity of the *Parthenium hysterophorus* extracts was assayed by Beta carotene bleaching well agar diffusion method. Flower extracts (chloroform and Ethyl Acetate) showed appreciable antioxidant activity in Beta-carotene bleaching assay. Rest of the flower fraction exhibited anti-bleaching activity in the normal range. Some of the root extracts (Acetone, ethanol, and aqueous) showed considerable activity. Rest of the root fractions exhibited moderate antioxidant activity. LC-MS analysis of the potent *Parthenium hysterophorus* extracts revealed the presence of various compounds having different molecular weight. The LC-MS scan demonstrated presence of different compounds showing 3–20 min retention time (RT).

IV. DISCUSSION

Development of multiple drug resistance in pathogens has given force to search new antimicrobial substances from alternate sources. There have been numerous mechanisms projected for the antimicrobial activity of effective drugs together with plant extracts [21]. In many cases phytochemicals can be more effective than chemically synthesized pure compounds because they are a complex mixture of components. Their complexity enables them to interact with multiple molecular targets and thus it becomes more difficult for target microorganisms to develop resistance because of multiple response sites [27]. The literature revealed that secondary metabolites such as alkaloids, tannins, flavonoids, and other phytochemicals are responsible for the

antimicrobial activities in higher plants [16, 28]. It is possible that flavonoids in *Parthenium hysterophorus* extracts and other group of phytochemicals as reported in our previous study [2, 4] may find their use as future antibacterial agents.

It has been found that antioxidants reduce molybdenum (VI) to green coloured molybdenum (V) complex in phosphomolybdenum assay. The molybdenum (V) complex shows absorption maxima at 695 nm [29]. Most of the *Parthenium hysterophorus* extracts such as stem extract showed appreciable antioxidant activity. The difference in AO capacity of different extracts may be attributed to differences in their chemical composition. Recent reports indicated that several bioactive compounds present in plants have strong antioxidant activity [18, 21]. It is well known that chromophores such as carotene have alternate double and single carbon-carbon bonds which are known as conjugated system. The electrons in the orbitals of the double bonds overlap, creating a system of delocalized electrons across a large part of the molecule. Carotenoids undergo bleaching when exposed to oxidizing species which involves the interruption of the conjugated double bond system by cleavage or by addition to one of the double bonds [30]. The results demonstrated that some of the flower (chloroform and ethyl acetate), and root (AC) extracts possess appreciable -carotene bleaching inhibition activity. This indicates that *Parthenium hysterophorus* extracts exhibit antioxidant potential by virtue of their radical scavenging activity. Polyphenolic contents of the extracts have been reported to function as good electron and hydrogen atom donors and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products [2, 14, 31]. This study experimentally proves that many extract fractions of flower, stem, and root extracts exhibited antimicrobial potential and also previously proven by the Shashank Kumar et al. 2014 [36].

LC-MS analysis of the *Parthenium hysterophorus* stem (Acetone), flower (ethyl acetate), and root (benzene) extract showed variation in separated compounds as indicated by different RT, peak area, and *m/z* values. Occurrence of the variable pattern of distribution of compounds may be responsible for the different extent of biological activities shown by the test extracts.

V. CONCLUSION

The study revealed that phytochemicals present in various parts of the *Parthenium hysterophorus* extracts exhibit biological properties. Many extract fractions of flower, stem, and root extracts exhibited antimicrobial potential. LC MS data indicated presence of many compounds in the extracts with different RT (3–20 min).

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