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EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *PARTHENIUM HYSTEROPHORUS* LINN.

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ABSTRACT

The most common detrimental weed *Parthenium hysterophorus* belonging to the family Compositae was evaluated in this study. The research works which were carried out during the past were mainly based on the control and elimination of this weed due to its noxious effect. However, this plant was selected for the present work to determine the antioxidant and antimicrobial properties present in it with the aim of finding modern drugs with less side effects. Phytochemical screening was carried out qualitatively by color reactions with different reagents. The antioxidant activity and antimicrobial activity were carried out by DPPH scavenging method and cup diffusion method respectively. The phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, terpenoids, tannins, saponins, cardiac glycosides and carbohydrates. The methanolic extract showed the moderate antioxidant activity with IC_{50} value of 81.43 µg/ml whereas the ethyl acetate and hexane acetate showed least antioxidant activity with IC_{50} value of 250.33 and 827.41µg/ml respectively. The ethyl acetate extract showed the potent activity against *Bacillus cereus* and moderate activity against *Staphylococcus aureus*, *Salmonella typhi* and *E. coli* whereas hexane and methanolic extract did not show antimicrobial activity against these organisms. The present study revealed the presence of high antimicrobial activity in ethyl acetate extract and moderate antioxidant activity in the methanolic extract of this plant. This study can be basis for the further research to find out more detail information regarding the relationship

between antioxidant activity and other quantitative phytochemical content which may help to highlight the chemicals which are responsible for this activity.

KEYWORDS: *Parthenium hysterophorus*, antimicrobial activity, antioxidant activity, DPPH free radical scavenging method, Cup diffusion method.

INTRODUCTION

Nepal lies between India on the South and China on the North. It occupies only small fraction of land but due to its unique geography and climate it is home to different variety of medicinal plants.[1] The land contains various types of aromatic plants having other non-medicinal values. The research of these plants has become an integral part to utilize them in an effective way to support the economy as well as medicinal need of the developing country and poor people of Nepal as well as people around the globe.[1] Among the Asian countries, the country Nepal is ranked in 9th position for its floral wealth. It has been estimated to have 9,000 species of flowering plants and 10% of species are reported as aromatic and medicinal plants. These plants part have been used in many purposes such as in medicines, food and in perfumes as well. For the first time, Pandey (1961) has reported 73 medicinal plants and aromatic plants (MAPs). After that, 483 species and 690 species of MAPs were reported by Department of Medicinal Plants (DMP, 1970) and Malla and Shakya (1984) respectively in Nepal. Further, ethno botanical information of 1,500 plant species were reported by Manandhar (2002) which consist of majority of plants having medicinal value.[2] There is a wide spread belief that herbal medicines are much more safer than synthetically derived drugs possessing side effects due to which there is great interest in this type of plant derived drugs.[3] Weeds are the plants known to have toxic and undesirable effect growing invasively in wrong places. Traditionally, this plant has been used by local people for treating many ailments.[4]

Parthenium hysterophorus, a poisonous and problematic weed is the most recent invaders in Kathmandu valley. It is the native of tropical and subtropical America belonging to the family Compositae. It is recorded in the global invasive species database and also known as the top ten weeds of the world. It can invade all types of land and can cause detrimental effect in the environment due to its potential to spread vigorously. It is also responsible for high losses in the yield crops, contact dermatitis, fever and respiratory malfunctions in human.[5] If the medicinal activity of such plant is explored properly then it could help in developing the

local economy of the nation and also aid in developing less expensive plant source for manufacturing medicines.

An antioxidant is a compound capable of inhibiting or slowing the oxidation process of other molecules by preventing the initiation and propagation of other oxidizing molecules. Oxidation is a chemical reaction in which the transfer of electrons from a substance to an oxidizing agent takes place. This type of reaction results in the formation of free radicals, which start chain reactions causing oxidative damage to the cellular components. The antioxidant molecules present in our body may be insufficient to block these free radicals. Nowadays, there has been an increase interest in area of "Natural antioxidants" from plant materials around the world for scientific research. Therefore, the search for natural antioxidant, especially those of plant origin, are becoming more and more popular in recent years all over the world.[6,7] Thus, antioxidant property in this plant is being studied.

The diseases which are infectious and parasitic are known as the second most dangerous cause of death after the cardiovascular diseases. This disease have resulted in 15.6% of the death in women and 16.7% of death in men causing 14 million death worldwide each year. Thus, the people around the world are facing problem due to this bacterial infections. The decrease in the efficacy of the antimicrobial medicine to treat the emerging infectious organisms with the potential of rapid global spread as well as antibiotic resistance is worsening this problem. As a result of this, there is a continuous focus on the development of newer and safer drugs having less side effects from plants commercially. These plant based antimicrobials have not been explored properly even though they have got tremendous therapeutic potential as well as immense impact in the treatment of infectious diseases.[8,9] Hence, this study was carried out to explore its antimicrobial property to tackle with ever increasing problem of antimicrobial resistance. Previous research has showed the significant activity of the methanolic extract of leaves of *Parthenium hysterophorus* against different microorganisms.[10] Another article showed the efficacy of petroleum ether extract of *Parthenium hysterophorus* against wide range of enteric bacterial pathogens.[11] *Parthenium* was also used against bacteria causing urinary tract infection according to previous studies of traditional uses. Hence, on the basis of these studies and researches, the extracts of this weed were tested against two gram positive bacteria: *Bacillus cereus*, *Staphylococcus aureus* and two gram negative bacteria: *Escherichia coli* and *Salmonella typhi*. These organisms are the

most prominent and their continual emergence as an antibiotic resistant are the main reason for choosing these organisms for present study.

MATERIALS AND METHODS

Study design

Experimental and descriptive research design.

Plant material

The sample required (aerial parts of *Parthenium hysterophorus*) was collected from Nakhu area of Kathmandu valley which stands at an elevation of approximately 1,400 meters (4,600 ft) in the month of June and July during rainy season and was identified as *Parthenium hysterophorus* at Pharmacognosy section, National Herbarium and Plant Laboratory, Lalitpur, Nepal.

Chemicals and apparatus

Hexane, ethyl acetate, methanol, diphenyl-1-picrylhydrazyl reagent (Hi-Media), ascorbic acid, ofloxacin, UV-Visible spectrophotometer (SHIMADZU), soxhlet apparatus (Borosil) and rotary vacuum evaporator (ATICO India).

Organisms

Pure bacterial culture of two gram positive organisms named *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* and two gram negative organisms named *E. coli* (ATCC 25922), *Salmonella typhi* were obtained from the Microbiology Laboratory of National College, NIST.

Plant processing

The plant material i.e. aerial parts of *Parthenium hysterophorus* was cut into pieces and shade dried at room temperature. Dried sample was crushed into powder by electric blender and subjected to extraction in soxhlet apparatus by using non-polar to polar solvents (hexane, ethyl acetate and methanol). The obtained extracts were dried by using rotatory evaporator drier under vacuum at temperature 40ºC till solid mass was obtained and thus obtained solid mass was poured into the glass vials. Then, the extracts were preserved in refrigerator at 4ºC for further analysis.

Physical evaluation

The ash value was determined by following the procedure of World Health Organization (WHO).[12] About 2g of the ground air-dried material of *Parthenium hysterophorus* was accurately weighed and was placed in a previously ignited and tarred crucible (usually of platinum or silica). The material was spread in an even layer and ignited at 570ºC until it was white, indicating the absence of carbon. Then it was cooled in a desiccator and was weighed. The content of total ash was calculated in percentage.

Phytochemical Screening

The phytochemical screening was done in different extracts of *Parthenium hysterophorus* which included qualitative tests by their color reaction with different reagents. Each extract was subjected to test for alkaloids, glycosides, cardiac glycosides, tannins, terpenoids, flavonoids, saponins, carbohydrates and proteins.

Antioxidant activities by DPPH Scavenging

The procedure for antioxidant activity was adapted from Pandey et al.[13] A stock solution of concentration 100µg/ml of different extracts of *Parthenium hysterophorus* as well as standard ascorbic acid were prepared. Sample solutions of ascorbic acid and plant extracts of concentration (5, 20, 40, 60, 80, 100µg/ml) were prepared from the stock solutions using methanol. Then, 0.1 mM solution of DPPH in methanol was prepared in a volumetric flask which was completely kept away from the light. After that, 1ml of this solution and 1ml of methanol was added to 1ml of each different concentration of sample plant extracts and ascorbic acid solutions. The mixture was kept in dark for 30 minutes. Similarly, as control, 1ml of 0.1mM DPPH was mixed with 2ml of methanol (solvent) and kept in dark for 30 minutes. The Absorbance of all the samples were measured using UV-Visible spectrophotometer at 517nm. The experiment was performed in triplicate. The mixture with lower absorbance indicated the presence of higher free radical scavenging activity. The DPPH radical scavenging activity was calculated using the following equation:

% Radical Scavenging = (Ao – AT) / Ao × 100.

Where, Ao = Absorbance of control

AT = Absorbance of test or standard

The IC_{50} values were calculated from linear regression by plotting the graph between concentration and % radical scavenging. The IC_{50} indicates the concentration of the sample required to scavenge 50% of DPPH free radicals.

Antibacterial activity of the extracts

Preliminary antimicrobial test of *Parthenium hysterophorus* was carried out by cup plate diffusion method. It was performed in three extracts of plant i.e. hexane, ethyl acetate and methanol of concentration 100mg/ml by dissolving in DMSO. Ofloxacin (50µg/ml) was used as reference standard antibiotic.

The extracts which showed antimicrobial activity, were then subjected for antimicrobial screening using reference standard by tube dilution method for determination of minimum inhibitory concentration (MIC). The antimicrobial test by cup plate diffusion method was carried out following the protocol of Joshi et al.[14]

Preparation of plant extracts

Antimicrobial screening was performed in the hexane, ethyl acetate, and methanolic extracts of the plant. DMSO was used as the solvent. 100mg of the extracts were dissolved in 1ml of 10% DMSO.

Preparation of standard

Ofloxacin was used as the standard antibiotic drug. 50µg of ofloxacin was dissolved in 1ml of distilled water.

Preparation of agar plates

Muller Hinton Agar plates were prepared from commercially available dehydrated base according to the method prescribed in agar pack. The media was sterilized in autoclave at 121ºC for 15 minutes. Then, the sterilized media was allowed to cool. 25ml of freshly prepared media was poured in sterilized petri dishes and left for solidification at room temperature.

Preparation of standard culture inoculums of test organism

Bacterial suspension was prepared by touching the top of isolated colonies from an agar plate culture and transferring into a tube containing Muller Hinton broth medium. The broth culture was inoculated at 35ºC for 2 hours until it achieved the turbidity of 0.5 McFarland standards, resulting a suspension containing approx. 1-2 × 10^8 CFU/ml.

Preparation of nutrient broth

Nutrient broth was prepared according to the method prescribed in commercially available pack. The media was sterilized in autoclave at 121ºC for 15 minutes. Then the sterilized media was allowed to cool.

Inoculation of test plates

After adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the suspension. The excess of the inoculum suspension from the swab was removed by rotating the cotton swab several times and pressing against the inside wall of the tube above the fluid level. The suspension was then swabbed on the dried surface of the agar plates by streaking the swab over the entire sterile agar surface. The plate was rotated approximately 60ºC to ensure an even distribution of inoculums and finally the rim of the agar was swabbed.

Preparation of cups in agar plate and reading of the plate

First of all, the plates were labeled properly with the help of permanent marker pen on the back side of the dish. Then, five cups of diameter 5mm were made at equidistance in the agar plates with the help of sterile cork-borer. The cups were filled with 50µl of 100mg/ml of each extract along with same volume of positive control i.e. standard antibiotic ofloxacin of concentration 50µg/ml and negative control i.e. DMSO.

The plates were left for some time to allow the diffusion of the extracts into the agar medium. All the plates were then incubated at 37ºC for 24 hours. After 24 hours of incubation, the plates were observed for the zone of inhibition and the diameter of the inhibition zone was measured using scale and the measurements were recorded in mm.

Determination of MIC values

In order to determine the MIC value, the tube dilution method was applied. For this, 13 clean sterile capped test tubes were taken and numbered properly with the help of permanent marker from 1 to 13. In the test tube no. 1, using a micropipette, 1000µl of crude extract and 1000µl of nutrient broth used as diluents were added. Then, 1000µl of nutrient broth was added to all other test tubes. The crude extract was diluted by two fold serial dilution method in 11 test tubes from test tube no. 1 to test tube no. 11. Then, 1000µl of diluted extract from test tube no. 11 was removed and discarded. The test tube no. 12 and 13 were used as positive control and negative control respectively. The crude extract was not added in negative control tube. 50µl of the tubes were incubated at 37ºC for 24 hours and were examined for visible

signs of bacterial growth. The lowest concentration (highest dilution) of antimicrobial agent preventing appearance of turbidity i.e. without growth is considered to be the minimal inhibitory concentration (MIC).

Statistical analysis

All the quantitative test was carried out in triplicate. Data obtained from the experiments was expressed as mean ± standard deviation (SD). Statistical difference of the results were evaluated using Microsoft excel 2007.

RESULTS

Physical evaluation

The total ash value of *Parthenium hysterophorus* was found to be 14.5%.

Extractive value

The extractive value of the powdered drug was higher for extraction with methanol, followed by ethyl acetate and hexane respectively.

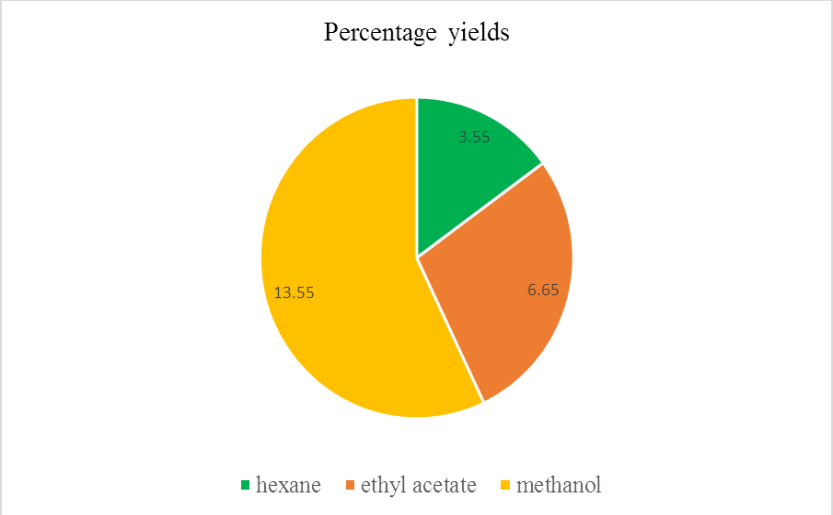

Figure 1: Pie-chart showing percentage yield of extraction of aerial parts of *Parthenium hysterophorus.*

Qualitative phytochemical screening

The qualitative phytochemical screening of the plant extracts by different reagents showed the presence of different group of active constituents in different solvent extracts as shown in Table 1.

Table 1: Phytochemical Screening of *Parthenium hysterophorus* extracts.

Tests/ Extracts	Hexane	Ethyl acetate	Methanol
Alkaloids	+	+	+
Glycosides	-	-	+
Cardiac glycosides	+	+	+
Tannins	-	+	+
Terpenoids	-	-	+
Flavonoids	+	+	+
Saponins	-	-	+
Carbohydrates	+	+	+
Proteins	-	-	-

Antioxidant activity

The results of antioxidant activity of plant extracts are shown below.

Table 2: Antioxidant activity of different plant extracts.

S.N	Concentration (µg/ml)	Percentage scavenging; Mean ± SD		
		Hexane extract	Ethyl acetate extract	Methanol extract
1.	5	2.45 ± 0.006	2.20 ± 0.003	33.33 ± 0.004
2.	20	2.69 ± 0.009	7.84 ± 0.002	36.76 ± 0.003
3.	40	2.94 ± 0.01	10.53 ± 0.002	40.19 ± 0.003
4.	60	3.92 ± 0.009	15.19 ± 0.008	43.87 ± 0.001
5.	80	8.08 ± 0.026	17.89 ± 0.0005	51.47 ± 0.003
6.	100	6.86 ± 0.015	20.83 ± 0.009	53.67 ± 0.027
IC50		> 100µg/ml	> 100µg/ml	81.43µg/ml

Table 3: Antioxidant activity of standard ascorbic acid.

S.N	Concentration (µg/ml)	Ascorbic acid
1	5	60.29
2	20	96.56
3	40	97.05
4	60	97.05
5	80	97.05
6	100	97.05
IC50		< 5µg/ml

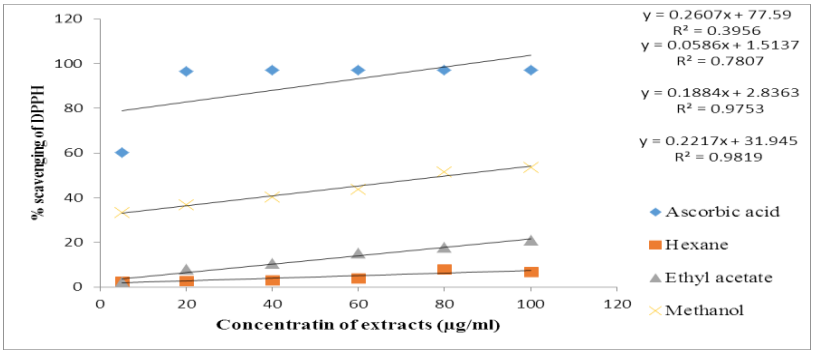

Figure 2: DPPH scavenging activity of different plant extracts and ascorbic acid.

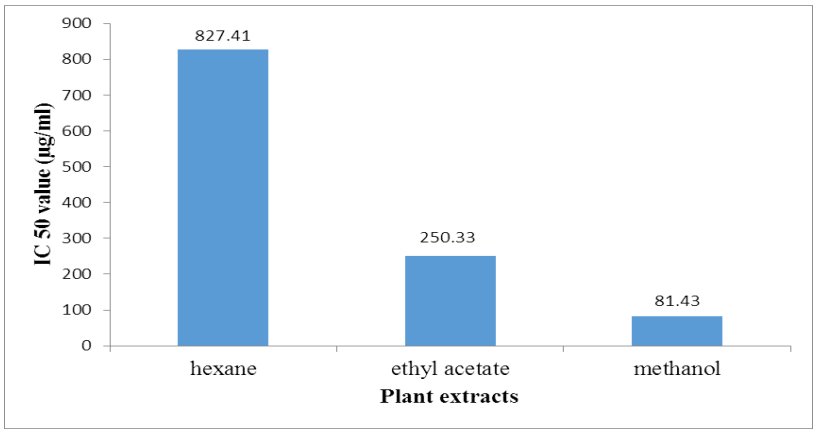

Figure 3: IC_{50} values of different plant extracts.

The antioxidant test for *Parthenium hysterophorus* showed the presence of moderate antioxidant activity in the methanolic extract and the least antioxidant activity in the ethyl acetate and hexane extracts. The IC_{50} values of the methanolic, ethyl acetate and hexane extracts were in the order of 81.43µg/ml > 250.33µg/ml > 827.41µg/ml respectively.

Antimicrobial activity of plant extracts

The result of preliminary study of antimicrobial activity of plant extracts is stated below. Ethyl acetate extract of the *Parthenium hysterophorus*showed the potent activity against *Bacillus cereus* and moderate activity against *Staphylococcus aurues*, *Salmonella typhi* and *E. coli*. However, hexane extract showed low activity against *Bacillus cereus* and did not show any antimicrobial activity against other three organisms. And, methanolic extract showed no antimicrobial activity against any organisms.

Table 4: Sensitivity of different extracts against different microorganisms.

Microorganisms	Zone of inhibition (mm)			
	Hexane extract	Ethyl acetate extract	Methanol extract	Standard*
Bacillus cereus	3mm	15mm	–	16mm
Staphylococcus aureus	–	9mm	–	19mm
Salmonella typhi	–	11mm	–	18mm
E. coli	–	10mm	–	29mm

Among three plant extracts, only ethyl acetate extract showed the antimicrobial activity against the sample microorganisms used.

"-" indicates No inhibition

Standard*= ofloxacin

Concentration of extract = 100mg/ml Concentration of standard = 50µg/ml.

The ethyl acetate extracts were then subjected to the minimum inhibitory concentration.

MIC was seen in test tube no.7 at the concentration 1.56mg/ml for *Bacillus cereus.*
MIC was seen in test tube no.4 at the concentration 12.5mg.ml for *Salmonella typhi.*

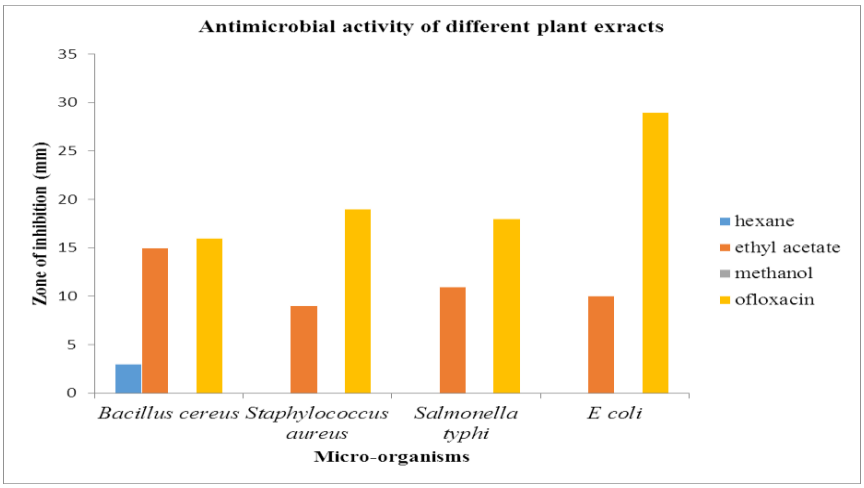

Figure 4: Antimicrobial activity of different plant extracts.

DISCUSSION

The present study was done for the evaluation of antioxidant and antimicrobial property of the worst and problematic weed *Parthenium hysterophorus*. This study was performed with the aim of developing the uses of this weed which could help to pave the path for indirect eradication of this weed with the target of utilizing it in a proper way to control its spreading.[15] The aerial parts of the plant were dried and grinded to get the powdered sample. The plant was then subjected for physical evaluation by determining the ash value which was found to be 14.5% which may be due to the presence of different types of inorganic materials such as heavy metals. This powdered plant material was then extracted with hexane, ethyl acetate and methanol successively by soxhlet apparatus. Each extract was subjected for phytochemical screening. Further studies were done on hexane, ethyl acetate and methanolic extract to carry out biological screening.

Preliminary screening is a very important step which helps to detect the valuable chemical constituents present in the plant. Qualitative phytochemical screening of the plant extracts showed the presence of different group of active constituents in different solvent extracts. Proteins showed the negative result for all solvent extracts. Methanolic extract showed the

presence of alkaloids, glycosides, cardiac glycosides, tannins, terpenoids, flavonoids, saponins and carbohydrates. Ethyl acetate extract was positive for alkaloids, cardiac glycosides, tannins, flavonoids and carbohydrates. Hexane showed the presence of alkaloids, cardiac glycosides, flavonoids and carbohydrates. Literature review regarding phytochemical screening of methanolic extract of leaves of *Parthenium hysterophorus* L. from Coimbatore, India found to contain Alkaloids, Glycosides, Phenols, Carbohydrates, Flavonoids, Cardiac glycosides, Saponins, Terpenoids, Tannins, Steroids and Protein. The Protein was present in the methanolic extract whereas it was absent in methanolic extract of present study.[10] The phytochemical screening of methanolic extract and hexane extract of *P. hysterophorus* from Pakistan showed the presence of alkaloids, flavonoids, saponins, terpenoids and cardiac glycosides in methanolic extract whereas cardiac glycosides and terpenoids were absent in *P. hysterophorus* n- hexane.[16] However, the present study revealed the presence of cardiac glycoside in hexane extract. The result from this study varied from the previous study. This may be due to variation in geographical distribution, time of plant collection, parts of plant collected and climatic factors.

The methanolic extract of aerial parts of *Parthenium hysterophorus* showed the moderate antioxidant property with IC_{50} value of 81.43µg/ml whereas the ethyl acetate and hexane extracts showed the least antioxidant property with IC_{50} value of 250.33 and 827.42µg/ml respectively. Ahmad, N., et al. (2011) in their research found the percentage free radical scavenging value of methanolic extracts of leaves of *Parthenium hysterophorus* to be 78.26% which is not similar to that of this study.[6] The antioxidant activity of aerial part of *Parthenium hysterophorus* was not found but the antioxidant activity of dried flower, leaves and roots were carried out.

The antimicrobial activity of ethyl acetate extract was found to be more potent in comparison to other extracts such as hexane and methanol. The methanolic and hexane extract of aerial parts of this plant did not show any antimicrobial activity. However, the previous study showed the methanol extract of leaves of *Parthenium hysterophorus* having antibacterial activity over different bacteria. Krishnavignesh, L. (2013) showed significant activity of methanolic extract of leaves of *Parthenium hysterophorus* against *Staphylococcus aureus* and *E. coli* with inhibition zone of 14mm and 13mm at the concentration of 1mg/ml and Fazal, H., et al. (2011) in their research have found inhibitory zone for methanolic extract of this plant to be 13±0.12 and 26±0.12 mm against *E. coli* and *S. aureus* respectively at the

concentration 15mg/ml.[10,17] The present investigation proved that the ethyl acetate extract possess promising antibacterial activity against *Bacillus cereus* with zone of inhibition of 15mm which is comparable to the zone of inhibition of positive control ofloxacin i.e. 16mm and have moderate antimicrobial activity against *Staphylococcus aureus* (9mm), *Salmonella typhi* (11mm) and *E. coli* (10mm) with zone of inhibition of ofloxacin at 19mm, 18mm and 29mm respectively. The variation in these activity of the plant from its previous studies may be due to difference in the time of collection, climatic change and environmental factors such as light, temperature, nutrition and humidity, geography and method of extraction.

CONCLUSION

The findings in the study revealed the presence of various phytochemicals such as flavonoids, alkaloids, glycosides, terpenoids, tannins, saponins, cardiac glycosides and carbohydrates. The methanolic extract showed moderate antioxidant activity with IC_{50} value of 81.43µg/ml. The ethyl acetate extract showed good antibacterial activity against various microorganisms. The present study provided the scientific evidence of its previous traditional use as antimicrobial agent. Therefore, it can be concluded that the weed, if explored properly, can result in discovery of new and alternate drugs. This plant showed promising antibacterial activity and moderate antioxidant activity. The further investigation on the relationship between antioxidant activity and other specific quantitative phytochemical test can be done to find out the components which are responsible for this activity. Hence, this study builds the platform for further research in the future for the discovery of modern drugs.

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