IJBPAS, November, 2022, 11(11): 5368-5375

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *PHYSALIS MINIMA* AND *LANTANA CAMERA* WILD MEDICINAL PLANTS

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Received 28th Dec. 2021; Revised 26th March 2022; Accepted 16th May 2022; Available online 1st Nov. 2022

https://doi.org/10.31032/IJBPAS/2022/11.11.6586

ABSTRACT

Medicinal plants have great value for the treatment and cure various diseases. Now scientific research has expanded our knowledge to discovered chemical composition and active constituents present in medicinal plants. Present research work was undertaken to the phytochemical, antioxidant and antibacterial activity of *Physalis minima* and *Lantana camera*. Phytochemical screening of all medicinal plants has been done with the use of solvent methanol, ethanol and water. Extracts of leaves were obtained by soxhlet extraction to find out the active constitution of plants. Phytochemical analysis of leaves extract has discovered the presence of medicinally important phytochemicals such as Saponin, Steroid, Tannin, Anthocyanin, Coumarin, Flavonoid, Diterpine, Phenol, Phlobatannin and Chalcone. The antioxidant of leaves extracts was assessed based on the radical scavenging effect of the stable 1,1-diphenyl-2-picryhydrazyl (DPPH). Antibacterial activity of aqueous and ethanolic extracts of *P. minima* and *L. camera* was studies for standard bacteria one Gram-positive (*Bacillus Subtilis*) and Gram-negative (*Escherichia coli*). The optimum inhibition zone size value for both the bacteria *Bacillus Subtilis* and *Escherichia coli* are 02 mm in *P.minima* and *L.camera*. The methanol and ethanol extracts of both plant show significant antioxidant and antibacterial activity. The

diversity of phytochemicals founds in *P. minima* and *L. camera* leaves could serve as a source of useful drugs.

Keywords: *P. minima, L. camera*, Phytochemical, Antioxidant, Antibacterial Activity

INTRODUCTION

Medicinal plants are plants that have all their parts leaves, stems, roots and flowers used for therapeutic purposes. Then desperately need to conservation of medicinal plants and cultivation of wild medicinal plants. Herbal wild medicinal plants are easily available, less expensive, no side effect and more efficient make them more attractive as therapeutic agents when compared to modern medicine **[1, 2]**. India has top ranked herbal medicinal producer because Indian plant biodiversity is the largest source of herbal plant medicine **[3]**. In the word there are 60 to 80 % of people in word use medicinal plants and their products for therapeutic purposes **[4]**. The medicinal value of these plants lies in the bioactive phytochemical constituents present in plants and that are beneficial to humans. Many active phytochemical like flavonoids, terpenoids, vitamins, alkaloids etc. were found to be responsible for these activities **[5]**. Present research work was undertaken on the phytochemical, antioxidant and antibacterial activity of Physalias minima and Lanthena camera. The methanol and ethanol extracts of both plants show significant antioxidant and antibacterial activity. The diversity of phytochemicals found in *P. minima* and *L. camera* leaves could serve as a source of useful drugs.

MATERIALS AND METHODS

Collection of plant materials *P. minima* and *L. camera* were collected from roadside area of near Lakhandur Tahsil of Bhandara district. The plant materials were identified by D. N. Lanjewar, Department of Botany, Yashwantrao Chawhan arts, commerce and science college Lakhandur. The *P. minima* and *L. camera* leaves was washed with tap water and used for the present study. Leaves were cut into small pieces, shade dried and ground to make fine powder. Process for Extraction 500 gm of each powder of the leaves were taken along with the 1000 ml of distilled water in a container. The mixture was shaken continuously with used of rotary shakers and place in a dark for 72 hour with occasional shaking. After 72 hour the mixture was filter and filtrate was concentrated to one third of the original amount. The resultant was used for phytochemical, antibacterial and antioxidant analysis **[6]**.

Phytochemical analysis

The ethanol extract of leaves of *P. minima* and *L. camera* was used for qualitative phytochemical analyses. Phytochemicals such as flavonoids, tannins, steroids, glycosides, saponins, phenolic compounds, terpenoids and alkaloids are analyzed **[5]**.

Antioxidant Activity By 1, 1-diphenyl-2-picrylhydrazyl (DPPH)

The antioxidant activity of the ethanol extracts of *P. minima* and *L. camera* leaves were assessed based on the radical scavenging effect of the stable DPPH **[7, 8]**. 0.005% of DPPH was prepared in ethyl alcohol and 4 ml of this DPPH solution was mixed with 4 ml of ethanolic plant extract solutions. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Visible spectrophotometer. 4ml ethanol with 0.005 DPPH solutions was used as blank. The optical density was recorded in spectrophotometer and % inhibition was calculated using the following formula.

Percentage (%) Inhibition of DPPH (% AA) = A – B × 100 /A

Where A=Optical density of the blank and B=Optical density of the sample.

Extraction concentration providing 50% inhibition IC_{50} values was calculated maximum and minimum values of %AA

Antibacterial activity (disk diffusion method)

Antibacterial activity was carried out to examine the sensitivity of some bacterial species against plant extracts of *P. minima* and *L. camera* leaves with a comparing the antibiotics for it by Disk Diffusion Method **[9]**. These bacteria included Gram-positive (*Bacillus Subtilis*) and Gram-negative (*Escherichia coli*).

RESULTS AND DISCUSSION

Phytochemical analysis

Preliminary screening of phytochemicals of ethanol extract of leaves of *P. minima* and *L. camera* are carried out as follows **[10-12]**.

Saponin:- 5 ml plants extract was mixed with 20 ml of double-distilled water then agitated in graduated cylinder For 15 min formation of foam indicates Saponin.

Steroid:- 1ml extract was dissolved in 10 ml of $CHCl_3$ and 1ml of concentrated H_2SO_4 acid was added from the side of a test tube. The upper layer turns red and the H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroids.

Tannin:- 4ml extract was treated with 4 ml Ferrous chloride formation of green color indicates that presence of condensed tannin.

Anthocyanin:- 2 ml of aqueous extract is added to 2 ml of 2N HCl & NH_3, the

appearance of pink-red turns blue-violet indicates the presence of anthocyanin.

Coumarin:- 3 ml of 2N NaOH was added to 2ml of aqueous extract formation of yellow color indicates coumarins.

Proteins:- Extract was treated with a few drops of concentrated nitric acid formation of yellow indicates the presence of proteins.

Flavonoid:- Extract was treated with 2N NaOH solution, formation of intense yellow color indicates presence of Flavonoid.

Diterpine:- Extract were dissolved in water and treated with 10 drops of $Cu(OAc)_2$ solution, formation of emerald green color indicates the presence of Diterpine.

Phenol:- Test extract were treated with 4 drops of Alcoholic ferrous chloride solution. Formation of bluish black color indicates the presence of Phenol

Phlobatannin:- when extract plant sample is boiled with dilute 0.1N HCl was taken red ppt was obtained as evidence for presence of Phlobatannin.

Chalcone:- 2ml of Ammonium Hydroxide was added to 0.5 ml ethanolic extract, the appearance of the red color showed the presence of Chalcone.

Carbohydrate:- Extract were dissolved individually in 5ml of distilled water and filtered. The filtrate was used for the following test. Filtrate was treated with 2 drops of alcoholic a-naphthol solution, the formation of violet ring at the junction indicates the presence of carbohydrates **(Table 1)**.

Table 1: Test of Phytochemical

Test of Phytochemical	*P. minima* Leaves	*L. camera* leaves
Saponin	+	-
Steroid	+	+
Tannin	+	+
Anthocyanin	+	+
Coumarin	+	+
Protein	-	+
Flavonoid	+	+
Diterpine	+	+
Phenol	+	+
Phlobatannin	+	-
Chalcone	-	+
Carbohydrate	+	+

Note: + = Present and - = Absent

Antioxidant Activity

The stock solution 1 mg/ml of ethanol extracts and DPPH solution was prepared. The required dilutions from 0.01 mg/ml to 0.1 mg/ml were prepared by appropriate dilutions **[7, 8]**. The optical densities of bank DPPH solution and sample solution can be calculate and found. With use of optical density of both solution percent antioxidant activities were calculated in **Table 2**.

Table 2: Optical Density and % Antioxidant Activity for Ethanolic Extract of P. minima leaves: (O.D. of Black DPPH = 0.585)

Conc.mg/ml	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
O.D. of P. minima	0.432	0.353	0.274	0.216	0.201	0.187	0.131	0.112	0.104	0.075
%AA P.minima	26.15	39.65	53.16	63.07	65.64	68.03	77.6	80.85	82.22	87.17

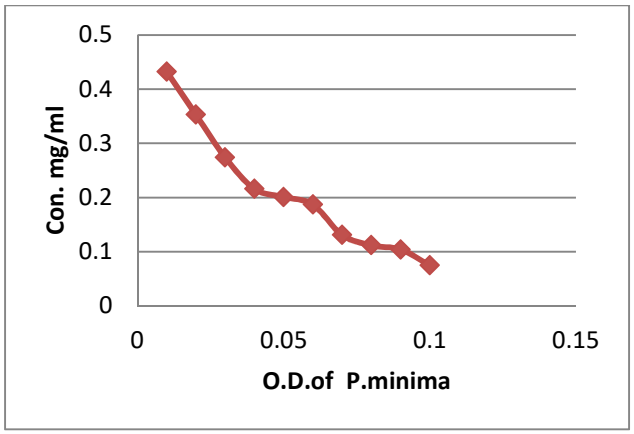

Figure 1: Decrease in Optical Density of Sample with Increase in Concentration of Ethanolic Extracts of P. minima leaves

Table 3: Optical Density and % Antioxidant Activity for Ethanolic Extract of *L. camera*: (O.D. of Black DPPH = 0.585)

Conc.mg/ml	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
O.D. of L. camera	0.412	0.345	0.261	0.214	0.198	0.179	0.127	0.107	0.095	0.042
%AA L. camera	29.57	41.02	55.38	63.41	60.15	69.4	78.29	81.7	83.76	92.82

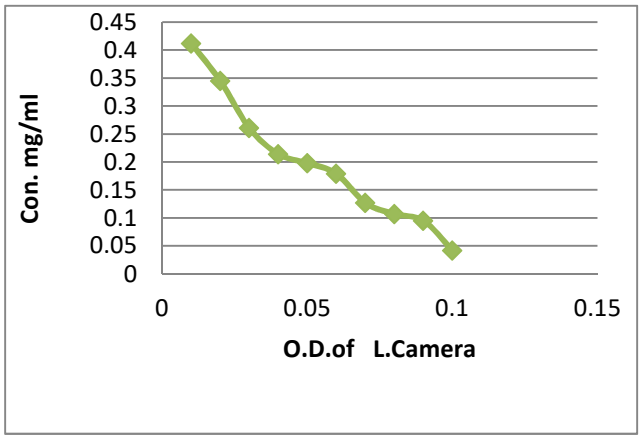

Figure 2: Decrease in Optical Density of Sample with Increase in Concentration of Ethanolic Extracts of *L. camera* leaves

Increase in Percent Antioxidant Activity with Increase in Concentration for ethanolic extract of *P. minima* leaves. Calculation of IC_{50} Value for *P. minima* leaves : = max – ½ (max-min)

= 87.17– ½ (87.17 – 26.15)

=56.66

IC_{50} value from graph corresponding ethanolic extract of P. minima leaves is 0.036 mg/ml.

Increase in Percent Antioxidant Activity with Increase in Concentration for ethanolic extract of *L. camera* leaves. Calculation of IC_{50} Value for *L. camera* leaves : = max – ½ (max-min)

= 92.82– ½ (92.82 – 29.57) =61.20

IC_{50} value from graph corresponding ethanolic extract of *L. camera* leaves is 0.052 mg/ml.

Antibacterial Activity:-

The examined bacterial species included Gram-positive (*Bacillus Subtilis*) and Gram-negative (*Escherichia coli*). Sterile discs 6 mm prepared from Whatman filter paper No. 1 were made to absorb 50 µg of the test samples **[13]**. Standard reference antibiotic discs (Nitrofurantoin 30 µg, and Nalidixic acid 30 µg) for bacterial species were used as positive control and solvent discs (Distilled water and Ethyl Alcohol) were used as negative control **[14]**. The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (1.5 x 108 cfu / mL)(15). Mueller-Hinton agar was prepared on the plates as the medium for the test organism **[16]**. The bacterial inoculums were spread evenly onto the surface of the agar plate using the sterile cotton bud and then the extracts discs, 20% DMSO impregnated discs and standard antimicrobial discs were situated on the inoculums agar superficial. The antimicrobial activity was interpreted from the size of the diameter of the zone of inhibition measured to the adjacent mm as experiential from a clear zone surrounding the disc.

Methanol and ethanol extract for *P. minima* and *L. camera* leaves are effective to antibacterial activity while aqua extracts are less effective. The optimum inhibition zone size value for both the bacteria *Bacillus Subtilis* and *Escherichia coli* are 02 mm in both plants. In case optimum inhibition zone size value of antibiotic Nitrofurantoin is 06 mm and Nalidixic acid is 5mm were the details results for antibacterial activity are shown as shown in **Table 4**.

Table 4: The effectiveness of three elements (Plant extracts, Antibiotics and bacteria)

Medicinal Plants		Bacterial Species	Antibiotic	Bacterial Species	Antibiotic
		Bacillus Subtilis (mm)	Nitrofurantoin (mm)	*Escherichia coli* (mm)	Nalidixic Acid (mm)
P. minima	Aqus Extr.	01	02	0.5	02
	Methanol Extr.	02	04	02	03
	Ethanol Extr.	02	05	01	04
L. camera	Aqus Extr.	0.5	01	0.25	01
	Methanol Extr.	01	04	01	05
	Ethanol Extr.	02	06	02	05

Legend: (mm) = Millimeter

CONCLUSION

- Phytochemical screening of selected wild medicinal plants clearly reveals that the maximum classes of photochemical are present in P.minima and L.camera
- The P.minima and L.camera Leave extracts demonstrate good DHHP radical activity with IC_{50} value for P.minima is 0.036 mg/ml and L. camera is 0.052 mg/ml which show good antioxidant activity.
- Leaves extract of P.minima and L.camera are exhibited significant antibacterial activity for bacterial species including Gram-positive (*Bacillus Subtilis*) and Gram-negative (*Escherichia coli*). The optimum inhibition zone size value for both the bacteria *Bacillus Subtilis* and *Escherichia coli* are 02 mm in P.minima and L.camera.
- Phytochemicals in plant extract serve as a source of drugs that are useful in the medicine of some diseases caused by bacteria and also as antioxidant agents.

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