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Research Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *PORTULACA QUADRIFIDA* LINN.

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ABSTRACT

Objective: The objective of the study was to investigate *in vitro* antimicrobial activity against enterotoxigenic *Escherichia coli* and *Bacillus subtilis* and preliminary phytochemical screening of the leaves of *Portulaca quadrifida* (Linn.).

Methods: The solvent extract such as petroleum ether, methanol, and water on the leaves of *P. quadrifida* (Linn) was prepared by Soxhlet extraction (continuous hot percolation method). These solvent extracts were screened for antimicrobial activity against enterotoxigenic *E. coli* and *B. subtilis* at various concentrations and were measured by observing zone of inhibition in mm by disc diffusion method (cup plate method).

Results: The preliminary phytochemical screening revealed the flavonoids, fats, and oils in all extracts. Similarly, the presence of alkaloids and tannins was obtained in the petroleum ether and methanolic extracts, while the presence of glycosides was obtained in the methanolic and water extracts. Further, proteins and sterols were found in petroleum extracts. The results of antimicrobial activity shown that methanolic extracts of the plant leaf showed good antimicrobial activity and petroleum ether and water extract showed similar activity but less antimicrobial activity than methanolic extract. The antimicrobial activities of extracts were compared with standard antibiotic such as chloramphenicol.

Conclusion: *P. quadrifida* (Linn.) has broad-spectrum antimicrobial activity and a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control. The phytochemical analysis of the crude extracts of this plant indicates the presence of major phytoconstituents which may have been responsible for the observed antimicrobial property.

Keywords: Phytochemical screening, *Escherichia coli*, *Bacillus subtilis*, Antimicrobial activity, Zone of inhibition.

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INTRODUCTION

After decades of serious thing with the modern medicinal system, people have started looking at the ancient medicinal systems such as Ayurveda, Siddha, and Unani. This is due to the adverse effects associated with synthetic drugs. Herbal drugs play an important role in health-care programs, especially in developing countries [1,3].

Portulaca quadrifida (Linn.), commonly known as Chicken weed, is an important herb belonging to the family Portulacaceae. The plant is a small, diffuse, annual, and erect herb. The plant is sour, bitter, hot, alternative, laxative; causes biliousness and "Kapha;" and cures fevers, asthma, cough, urinary disorders, inflammations, good for eye diseases, skin diseases, and ulcers. In Indo-China, the juice of the leaves is applied to abscesses and used as a collyrium; a decoction is given in dysentery [2,3]. In Nigeria, the leaves are used as a local application to swellings [5].

A traditional medicinal plant has been used in the Ayurvedic system of medicine for centuries, valued for its benefits in the management of urinary and inflammatory disorders [3]. The juice of the leaves is applied to abscesses and decoction is given in dysentery. The decoction of the plant can act as anthelmintic and used in the treatment of stomach complaints and gonorrhea [2]. *P. quadrifida* has been reported to possess antifungal activity [5].

The effect of the ethanolic extract of *P. quadrifida* Linn. in central and peripheral nervous system was studied using natural motor activity, *in vivo* muscle relaxant activity (grip strength), and anticonvulsant activity, and it is also found to have a good effect on central nervous system [3].

The therapeutic efficacy of *P. quadrifida* is extensively used in the Indian System of Medicine which has been established through modern testing and evaluation (pre-clinical and clinical trials) in different disease conditions. These studies establish the drug in the development for the treatment of various diseases such as neurological problems, convulsion, inflammation, dysentery, gastrointestinal disorders, and in case of some carcinogenic diseases also [4].

METHODS

Collection, identification, and extraction of plant materials

The leaves of plant *P. quadrifida* Linn. were collected from the Hatwalan village, Daund, Maharashtra, India, in the month of April to June. The leaves of the plant were plant authenticated by the botanist of the Botanical Survey of India, Pune. The cleaned materials were dried under shade with temperature ranging in between 18 and 32°C in a well-ventilated room and grounded to fine powder. The powdered *P. quadrifida* Linn. was sequentially extracted by Soxhlet extraction with solvents of increasing polarity such as petroleum ether (60–80°C), methanol (90–100°C), and water (100–120°C). After extraction, the extracts were removed and dried completely. The percentage yields were determined all extracts. All crude extracts were then subjected to phytochemical screening and antimicrobial tests.

Phytochemical screening

All crude extracts of *P. quadrifida* were subjected to a preliminary phytochemical screening. The presence of phytoconstituents was determined by the standard qualitative methods [6,7,10-12].

Antimicrobial activity [8-12]

Cup plate diffusion methods are mostly used for the investigation of antimicrobial activity of plant extracts. The cup plate method is easy to note the result and needs a small amount of extract.

Cup plate diffusion method

All the glassware and the Petri plates were sterilized by dry heat in an oven at 160°C for 1 h. Nutrient agar was prepared in distilled water. The nutrient agar was poured in sterile Petri plates aseptically and allowed to solidify at room temperature. All the Petri plates were aseptically flooded with 0.1 ml of the standardized culture. The holes of 7 mm were bored aseptically using sterile cork borer. The agar plugs were taken out carefully so as not to disturb the surrounding medium. The holes were filled completely with desired extract and kept in an incubator at 30°C for 48 h. After this, the Petri plates were observed for the antimicrobial activity and zone of inhibition was measured. The solvent effect was neutralized.

Preparation of inoculums

The suspension of all organisms was prepared by inoculating one colony of nutrient broth in a colony of strain in 15 ml of nutrient broth in a conical flask and incubated at 37°C for 24 h to activate the strain.

Bacterial culture

- *Bacillus subtilis* (ATCC 441) Gram-positive
- *Escherichia coli* (ATCC 25922) Gram-negative.

Composition of nutrient agar media

- Yeast extract - 10 g
- Peptone - 10 g
- Sodium chloride - 5 g
- Agar - 20 g
- Distilled water - 1000 ml.

Procedure for performing the cup plate diffusion method

Plates are prepared with the nutrient agar medium of about 4 mm layers. Different dilutions of petroleum ether extract (25 mg/ml–200 mg/ml), methanolic extract (25 mg/ml–200 mg/ml), and water extract (25 mg/ml–200 mg/ml) were carried out. Blank readings of the extracts were taken. Sterile, non-toxic cotton swab on a Wooden Applicator dipped in prepared inoculum and rotated soaked swab firmly against the upper inside wall of the test tube. Streak the entire agar surface of the plate with the swab 2–3 times, turning plate at 60^0 angles between each streaking. The inoculum allowed to dry for 5–15 min with lid in place. Properly bored the plate with borer and the disc is applied to standard drug. Chloramphenicol (30 mcg/disc) was used for standard antibiotics for the activity being most resistance in both Gram-positive and Gram-negative species and inhibits bacterial protein synthesis by binding to the subunit of the ribosome.

A zone of inhibition measurement

The antimicrobial activity was recorded by measuring the width of the clear inhibition zone around the disc using a zone reader (zone size interpretative scale).

RESULTS

Yield of various extracts

The percentage yield of the three extracts (petroleum ether, methanol, and water) of *P. quadrifida* is summarized in Table 1. The yield of the various extracts depends on the type of solvent used in the extraction procedure.

Phytochemical screening

The phytochemical screening of petroleum ether, methanol, and water extracts of *P. quadrifida* was carried out by standard procedures, and the results are summarized in Table 2. According to the observed results, the flavonoids, fats, and oils were presented in all extracts. Similarly, the

Table 1: Percentage yield of various extracts from different solvents of *Portulaca quadrifida*

Solvent used	Color	Yield obtained (g)	Yield obtained (% w/w)
Petroleum ether	Pale yellowish brown	1.59	1.35
Methanol	Dark green	1.80	1.53
Water	Dirty brown	1.75	1.49

Table 2: Phytochemical screening of various extracts of *Portulaca quadrifida* leaves

Tests	Petroleum ether extract	Methanolic extract	Water extract
Carbohydrates			
Molisch's test	Negative test	Negative test	Positive test
Fehling's test	Negative test	Negative test	Positive test
Proteins			
Biuret test	Positive test	Negative test	Negative test
Xanthoproteic test	Positive test	Negative test	Negative test
Amino-acids	-	-	-
Ninhydrin test	Negative test	Positive test	Positive test
Fats and oils			
Solubility test	Positive test	Positive test	Positive test
Steroids			
Salkowski reaction	Positive test	Negative test	Negative test
Liebermann's reaction	Blue coloration	No blue coloration	No blue coloration
Volatile oil			
Odor	Characteristics	No specific	No specific
Staining	Permanent stains	Permanent stains	Permanent stains
Solubility	Soluble	Soluble	Soluble
Glycosides			
Cardiac (Killar-killani)	Positive test	-	Positive test
Anthraquinone (Borntragers)	Negative test	Positive test	Positive test
Saponin (foam)	-	-	Positive test
Coumarin color	Negative test	Positive test	Positive test
Flavonoids			
Lead acetate test	Positive test	Positive test	Positive test
Tannins			
5% $FeCl_3$ solution+extract	Positive test	Positive test	Negative test
Alkaloids			
Murexide test (purine alkaloids)	Negative test	Positive test	Positive test

Table 3: Zone of inhibition of petroleum ether extract of *Portulaca quadrifida* plant with standard against bacteria

Extract (mg/ml)	Zone of Inhibition (mm of diameter)	
	Bacillus subtilis	*Escherichia coli*
Petroleum ether extract		
Blank	--	--
25	--	--
50	--	--
100	5	--
150	7	5
200	10	7
Chloramphenicol (standard)	15	10

Table 4: Zone of inhibition of methanolic extract of *Portulaca quadrifida* plant with standard against bacteria

Extract (mg/ml)	Zone of inhibition (mm of diameter)	
	Bacillus subtilis	*Escherichia coli*
Methanolic extract		
Blank	--	--
25	--	--
50	05	--
100	07	05
150	10	07
200	15	10
Chloramphenicol (standard)	20	15

Table 5: Zone of inhibition of Water extract of *Portulaca quadrifida* plant with standard against bacteria

Extract (mg/ml)	Zone of inhibition (mm of diameter)	
	Bacillus subtilis	*Escherichia coli*
Water extract		
Blank	--	--
25	--	--
50	--	--
100	05	--
150	07	03
200	10	07
Chloramphenicol (standard)	12	10

presence of alkaloids and tannin was observed in the petroleum ether and methanolic extracts, while the presence of glycosides was observed in the methanolic and water extracts. Further, proteins and sterols were found in petroleum extracts. The results obtained were compared and satisfied with the standard literature.

Antimicrobial activity

Antimicrobial activity of *P. quadrifida* was carried out by cup plate diffusion technique on different concentrations of petroleum ether extract (25–200 mg/ml), methanolic extract (25–200 mg/ml), and water extracts (25–200 mg/ml) and was comparable with the standard. A maximum zone of inhibition was found in the methanolic extract. A zone of inhibition of petroleum ether and water extract is similar but less than methanolic extract. The results obtained are summarized in Tables 3-5 and Figs. 1-3.

DISCUSSION

This study deals with the leaves of *P. quadrifida*, a medicinal herb. According to this study, methanolic extract of this plant parts was found to be certainly much better than other extracts in the percentage yield. This may be due to the better solubility of the active constituents in methanol solvent.

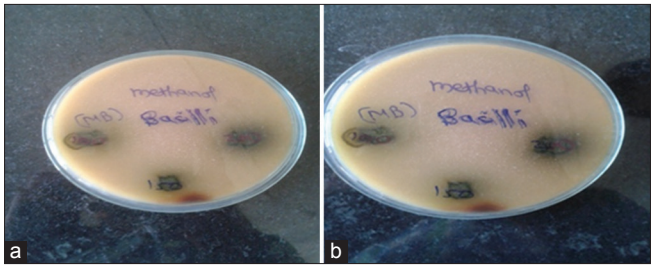

Fig. 1: (a and b) Zone of Inhibition of methanolic extract on *Bacillus subtilis*

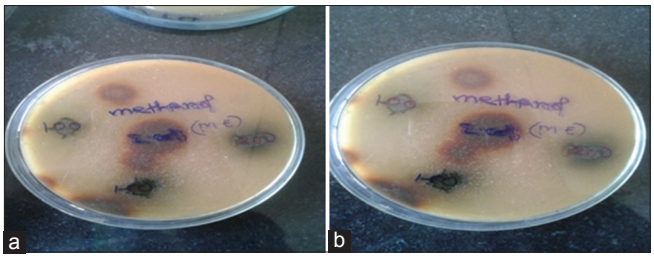

Fig. 2: (a and b) Zone of inhibition of methanolic extract on *Escherichia coli*

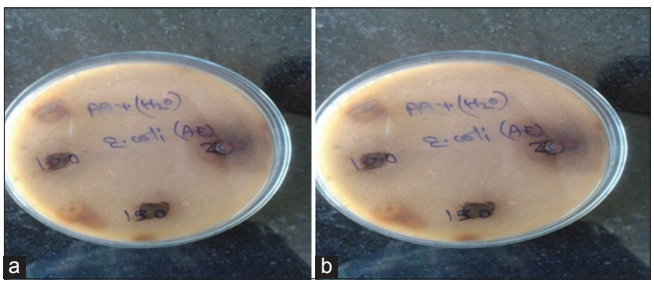

Fig.3: (a and b) Zone of inhibition of water extract on *Escherichia coli*

Phytochemical screening of the plant sample is very important parameter because it provides wide information for the therapeutic agent discovery and finds the new source of economical compounds such as alkaloids, flavonoids, saponins, and tannins [4]. These studies establish the drug in the development for the treatment of various diseases such as neurological problems [15], convulsion [16,17], inflammation [2], dysentery [5], and gastrointestinal disorders [16] This plant also possesses pharmacological activities such as antimicrobial [11,12] and antifungal properties [5]. Therefore, the leaves of *P. quadrifida* are important as it possesses various biological activities and medicinal properties due to the presence of various phytoconstituents.

The emphasis was given on *in vitro* antimicrobial studies on leaves of *P. quadrifida* to find their usefulness to human being. This plant was collected from the Hatwalan village, Daund, Maharashtra, India. The herbarium of the plant specimen was deposited at the Botanical Survey of India, Pune. Antimicrobial activity was performed of two strains of microorganisms, in which *B. subtilis* (Gram-positive) and *E. coli* (Gram-negative) strains are studied. Antimicrobial activity was performed by cup plate diffusion technique, and different concentrations of petroleum ether, methanol, and water extract (25–200 mg/ml) were prepared. Methanolic extract shows good antimicrobial activity at 200 mg/ml concentration where chloramphenicol was used for standard antibiotics for activity. Methanolic extract is more potent toward Gram-positive bacteria.

CONCLUSION

Leaves of *P. quadrifida* Linn., on the phytochemical analysis, shows the presence of alkaloids, amino acids, proteins, flavonoids, saponins,

sterols, and glycosides which have the highest medicinal value. The high zone of inhibition was seen in the methanolic extract which indicates the high antimicrobial activity than the other two extracts. The methanolic extract containing tannins, glycosides, and flavonoids which may has been responsible for the observed antimicrobial activity.

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AUTHORS' CONTRIBUTIONS

The design of the work and correction of the manuscript were done by the second author Dr. K.N. Gujar. The experimental part of the work was done by first and third authors Mrs. S.G. Jagtap and Mr. Aniket Ghare. The writing of the manuscript was done by first author Mrs. S.G. Jagtap.

CONFLICTS OF INTEREST

The authors declare no conflicts of interests.

REFERENCES

1. Pandey MM, Rastogi S, Rawat AK. Indian traditional ayurvedic system of medicine and nutritional supplementation. Evid Based Complement Alternat Med 2013;2013:376327.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Allahbad: Lalit Mohan Basu, Publishers; 1984. p. 72-7.
3. Patil AG, Joshi VS, Koli SP, Patil D. Pharmacognistical and phytochemical analysis of *Portulaca quadrifida* Linn. Res J Pharm Biol Chem Sci 2012;3:90-100.
4. Syed KM, Ahmed LT, Paramjyothy S. J Appl Pharm Res Pharm Technol 2010;2:1386-90.
5. Hoffman BR, Alas D, Blanco K, Wilder Hold N, Lewis RE, Williams L. Pharm Biol 2004;42:3-17.
6. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. 21st ed. Pune: Nirali Prakashan Publishers; 2011. p. 10.1-10.4.
7. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 46th ed. India: Nirali Prakashan Publishers; 2007.
8. Kokare CR. Pharmaceutical Microbiology Experiments & Techniques. 2nd ed. Nashik, India: Career Publishers; 2007. p. 55-7.
9. Kokare CR. Pharmaceutical Microbiology & Biotechnology. 3rd ed. Pune: Nirali Prakashan Publishers; 2006. p. 21.1-21.6.
10. Bashyal S, Guha A. Evaluation of *Trachyspermum ammi* seeds for antimicrobial activity and phytochemical analysis. Asian J Pharm Clin Res 2018;11:274-7.
11. Eltayeb IM, Abdeen Salim Hamid A. Phytochemical screening and antimicrobial activity of *Thuja occidentalis* seeds extracts against the isolated common skin infecting microorganisms. Int J Pharm Pharm Sci 2017;9:131-4.
12. Vadnere Gautam P, Rageeb Usman Md, Lodhi S, Vaishali P. Phytochemical investigation and *in vitro* antimicrobial screening of *Santalum album* seeds extracts. Int J Pharm Pharm Sci 2017;9:117-24.
13. Das M, Ashok Kumar D. Phyto-pharmacological review of *Portulaca quadrifida* linn. J Appl Pharm Res 2013;1:1-4.
14. Goel RK, Sairam K, Babu MD, Tavares IA, Raman A. *In vitro* evaluation of Bacopa monniera on anti-*Helicobacter pylori* activity and accumulation of prostaglandins. Phytomedicine 2003;10:523-7.
15. Kamil M, Liyakha T, Md Ahmed LT, Paramjyothi S. Neuropharmacological activity. Int J Pharm Technol Res 2010;2:1386-90.
16. Dar A, Channa SJ. Gastrointestinal disorders. Ethnophamacol 1999;66:167-74.
17. Parmart NS, Prakash S. Screening Methods in Pharmacology. New Delhi: Narosa Publishing House; 2006. p. 90-1.