PATNA WOMEN'S COLLEGE 1940

IRIS
Journal for Young Scientists
ISSN 2278 – 618X **(Print)**
ISSN 2278 – 6384 **(Online)**

http://www.patnawomenscollege.in/journal

Analysis of phytoconstituents present in the leaf extract of *Trianthema portulacastrum* and its antimicrobial assay

• **Surabhi Dutta** • **Shweta Jha** • **Kirti Kashyap**
• **Urvashi Sinha**

Received : November 2016
Accepted : March 2017
Corresponding Author : Urvashi Sinha

Abstract : *The present study dealt with the phytochemical screening, estimation, antioxidant activity and antimicrobial potential of the leaf extract prepared in distilled water and methanol of Trianthema portulacastrum. The aqueous extract showed the presence of Flavonoid, Tannin, Protein, Terpenoid,Carbohydrate and methanolic extract showed the presence of Phenol, Tannin, Protein, Carbohydrate, Alkaloid. Among all the phytoconstituents detected, Protein was 840g/m, and Carbohydrate was 80µg/ml. Functional group were detected by using FT-IR.The antioxidant property was evaluated using hydrogen peroxide scavenging assay and the percentage inhibition was calculated as 5.031 in aqueous and 9.129 in methanol. The antibacterial assay of methanolic leaf extract showed partial effectiveness against the pathogenic bacterial strain Escherichia coli and both the leaf extracts were found to be ineffective against the fungal strain Aspergillus niger.*

Keywords: *Phenol, Tannin, Protein, Carbohydrate, Escherichia coli, Aspergillus niger.*

Surabhi Dutta
B.Sc. III year, Botany (Hons.),
Session : 2014-2017, Patna Women's College,
Patna University, Patna, Bihar, India

Shweta Jha
B.Sc. III year, Botany (Hons.),
Session : 2014-2017, Patna Women's College,
Patna University, Patna, Bihar, India

Kirti Kashyap
B.Sc. III year, Botany (Hons.),
Session : 2014-2017, Patna Women's College,
Patna University, Patna, Bihar, India

Urvashi Sinha
Asst. Prof., Deptt. of Botany,
Patna Women's College, Bailey Road,
Patna – 800 001, Bihar, India.
E-mail : .urvashi_vrm@yahoo.co.in

Introduction:

Trianthema portulacastrum, also known as Bishkhapra, Horse purslane, Gadabani and Lalsabuni is one of the most common weed of family Aizoaceae (Singh et al, 1982) .Two forms are reported to occur in this species, a red –colored form known as Lalsabuni and a green colored form known as Svetsabuni which has green stem and white flowers. It grows abundantly in Bihar, Uttar Pradesh and West Bengal. This is not cultivated commercially but is found as a tropical problematic terrestrial weed by virtue of its infestation in plains, river beds and in waste lands. Flowers bloom in the month of February to October. The plant is used as a herb which is found on the ground in a circle and

4-6 feet of length and is used in ayurvedic medicine. The plant has been found useful in the treatment of heart diseases, anemia, inflammations, pikes and astices. The plant has been used in the indigenous system of medicine for the obstruction of the liver asthma, amenorrhea, dropsy, edema and beriberi. It also acts as antidotes to alcoholic person. In this plant, photochemicals are present which accumulate in different parts of the plant, such as in the stem, leaf and root (Shanmugam et al (2006). These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antifungal activity (Nawaz et al, 2003) molting hormone activity, antimicrobial activity and anticarcinogenic activity (Tripathi, 2004). Bishkhapra (*Trianthema portulacastrum* Linn.), belonging to the family Aizoaceae, has enormous traditional uses against diseases and some bioactive compounds have been isolated from this weed. Therefore, this plant was selected for antimicrobial assay.

Materials and Methods:

The fresh leaves of *Trianthema portulacastrum* were collected from the campus of Patna Women's College. The fresh leaves were cleaned and dried in hot air oven at 45 The dried leaves were ground to a fine powder in a blender.It was then dissolved in distilled water and methanol in 10:1 (v/w) (Saklani et al 2011).The mixture was centrifuged at 2500rpm for 10 minutes. The filtrate was obtained was used as final extract and was subjected to differentphytochemical tests for detection, analysis and assessment of primary and secondary metabolites, antioxidant and antimicrobial properties.

The aqueous and methanolic extract of leaves of *Trianthema portulacastrum* were subjected to preliminary phytochemical testing for the detection of major chemical groups following Manjunath et al., (2006). To test the presence of carbohydrate. Devmurari and Jivani (2010) was followed. The details of the tests are as follows:

One ml of aqueous and methanolic extract was taken in test tubes separately and 2 drops of concentrated H_2SO_4 was added in each test tubes. Formation of Crimson colour is the indication of presence of Flavonoid.

One ml of aqueous and methanolic extract was taken in test tubes separately and treated with Mayer's reagent. Formation of cream precipitate is the indication of presence of alkaloid.

One ml of aqueous and methanolic extract was taken in test tubes separately and few ml of ferric chloride solution was added. Formation of Dark green colour is the indication of presence of Phenol.

One ml of aqueous and methanolic extract was taken in test tubes separately and 2ml of chloroform along with 3ml of conc. Sulphuric acid was added. Formation of reddish brown colour is the indication of presence of Terpenoid.

One ml of aqueous and methanolic extract was taken in test tubes separately and few drops of ferric chloride solution were added. Formation of Black precipitate is the indication of presence of Tannin.

One ml of aqueous and methanolic extract was taken in test tubes separately and 2-3 drops of 1% alcoholic α-napthol solution along with 2ml of conc. Sulphuric acid was added. Appearance of violet colour ring is the indication of presence of carbohydrate.

One ml of aqueous and methanolic extract was taken in test tubes separately and 1ml conc. Nitric acid wasadded. A white precipitate was obtained. The solution was heated for 1 minute and cooled under tap water. It was made alkaline using excess of 40% sodium hydroxide. Formation of orange precipitate is anindication of presence of Protein.

Protein estimation was done by following the method of Lowry et al (1951). For carbohydrate and phenol methods of Dubois et al (1956) was followed.

Presence of functional group in the sample was detected by FTIR.The IR spectrum of the methanol and aqueous extract of *Trianthema portulacastrum* was recorded in a FTIR Spectrophotometer using KBr pellet method.

The leaf extracts was subjected to antibacterial test by disc diffusion method. For this study Pure culture of a pathogenic strain *Escherichia coli* was taken. Nutrient agar was poured on 6 sterilized petriplates. In an opaque vial, 5ml of saline water was taken and sterilized by autoclaving at 121°C and 15lbs/inch^2 pressure for 15 minutes. The loop, full of isolated bacteria were transferred into the opaque vials and mixed well using vortex to develop turbidity. With the help of sterilized pipette, 0.1ml of each isolate was pipetted out and dropped on the plate. It was then evenly spread over the media with the help of sterile glass spreader. The plates were kept at room temperature for 15minutes to allow surface moisture to be absorbed before loading the impregnated discs. Then, 12 discs were loaded 6 containing aqueous extract and rest 6 methanolic extract by means of sterile forceps, strictly under aseptic condition. The discs were placed on the plate. Then within 15 minutes discs were loaded, the plates were placed in incubator at 36ÚC for 24 hours following Tiwari et al (2011).

The leaf extract was subjected to antifungal test by disc diffusion method. For this study pure culture of a pathogenic strain *Aspergillus niger* was taken. Potato dextrose agar was poured on 5 sterilized petriplates. In opaque vials 5ml of saline water was taken and sterilized by autoclaving at 121°C and 15lbs/inch^2 pressure for 15 minutes. The loop, full of isolated bacteria were transferred into the opaque vials and mixed well using vortex to develop turbidity. With the help of sterilised pipette, 1ml of each isolate was pipette out and dropped on the plate. It was then evenly spread over the media with the help of sterile glass spreader. The plates were kept at room temperature for 15minutes to allow surface moisture to be absorbed before loading the impregnated discs. Then, 12 discs were loaded 6 containing aqueous extract and rest 6 methanolic extract by means of sterile forceps, strictly under aseptic condition. The discs were placed on the plate. Then within 15 minutes discs were loaded, the plates were placed in incubator at 27°C for 48 hours following Tiwari et al (2011).

Results and Discussion:

The phytochemical screening of the aqueous extract of the leaf revealed that all the major constituent responsible for nutritional and antinutritional factors were present in the leaf extract, as shown in Table 1.

Table 1. Phytochemical screening of leaf extract of *Trianthema portulacastrum*

S. NO.	PHYTOCHEMICAL CONSTITUENTS	AQUOUS EXTRACT	METHANOLIC EXTRACT
1.	FLAVANOID	+	_
2.	ALKALOID	_	+
3.	PHENOL	_	+
4.	TERPENOID	+	_
5.	TANNIN	+	+
6.	PROTEIN	+	+
7.	CARBOHYDRATE	+	+

Present (+), Absent (-)

It shows the presence of flavonoid, terpenoid, tannin, protein and carbohydrate in aqueous extract and the presence of alkaloid, tannin,phenol,protein and carbohydrate in the methanolic extract is similar to that obtained by the result of Devmurari and Jivani (2010).

The absorbance of protein, carbohydrate and phenol is shown and its in Table 2.

Table 2. Protein, Carbohydrate and Phenol present in leaf extract of *Trianthema portulacastrum*

S. No.	Phyto-compounds	Wavelength (nm)	Absorbance	Concentration (mg / ml)
1.	Protein	600	0.887	840
2.	Carbohydrates	490	0.921	80
3.	Phenols	765	0.428	420

Concentration of proteins was found to be highest whereas phenol concentration was the least which was similar to the result reported by Freeze (1998), Thomsen et al. (1991) and Vijayvergia and Kumar (2007).

Table 3. Antioxidant activity of leaf extract of *Trianthema portulacastrum*

S. No.	Sample	Absorbance of control	Absorbance of sample	% of inhibition
1.	Aqueous extract	3.189	2.311	5.031
2.	Methanolic extract	4.688	4.260	9.129

The leaf sample of *Trianthema portulacastrum* showed antioxidant activity (Table 3). In aqueous extract, the percentage of inhibition was 5.031 and in the methanolic extract, the percentage of inhibition was 9.129.

Table 4. Antifungal activity of leaf extract if *Trianthema portulacastrum*

S. No	Inoculum	Pathogenic Strain	Control	Zone of Inhibition		
				Aqueous Extract	Methanolic Extract	Control
1.	Bacteria	*Escherichia coli*	Chloram-phenicol	–	±3mm	±14mm
2.	Fungi	*Aspergillus niger*	Fucazole	–	–	+

Table 4 shows that the methanolic leaf extract of *Trianthema portulacastrum* was ineffective against the pathogenic fungi *Aspergillus niger* and effective against the bacteria *Escherichia coli*. However, aqueous extract was ineffective against pathogenic fungi *Aspergillus niger* and bacteria *Escherichia coli* both. Tiwari *et al* (2011) in his work has reported the bio efficacy of the leaf extract in methanol to be more effective against both bacteria and fungi.

The FTIR peaks at 1632.9 showed the presence of C=C (alkene), 1384.09 showed the presence of $–CH_3$,(alkane), 1116.35 indicates the presence of –OH group and 552.07 indicates presence of –C-Br(alkyl halide) in aqueous extract and in methanol extract 1633.73 showed the presence of C=C,1589.58 showed the presence of NH_2(amide),1384.05 showed the presence of CH_3,1350.13 showed the presence of C-F,513.72 showed the presence of C-Br.

Conclusion:

The present research work scientifically showed that the leaves of *Trianthema portulacastrum* is a potential source of vital constituents like alkaloid, flavonoid, phenol, terpenoid, tannin, carbohydrate and protein. Inhibition of bacterial and fungal growth by the leaf of *Trianthema portulacastrum* make them a cheap anti-bacterial and anti-fungal agent. The leaf also shows antioxidant activity which might be concluded that the hepatoprotective action of extract is due to its antioxidant activity. Hence, more research work needs to be done in this field to bring forth the latent potential of the leaf of *Trianthema portulacastrum* which may prove to be as useful in medicine.

As it is a weed, widely available in all the seasons, drastic conditions and ease of collection in low cost, the plant could serve as a “lead” for drug discovery that can be used for treating infectious diseases.

Acknowledgement:

We express our great sense of respect and gratitude to our Principal, Dr. Sister Marie Jessie A.C, for giving us the opportunity to do this research work. We are also grateful to Dr. Pinky Prasad, Head of the Department of Botany, Patna Women's College, Patna.

References:

Balyan RS, Bhan VM (1986). Emergence, growth and reproduction of horse purslane (*Trianthema portulacastrum*) as influenced by environmental conditions. *Weed Science*, 34:516–9.

Council of Industrial and Scientific Rearch (1976) New Delhi. *Wealth of India (Raw Materials),* Vol.10.p. 281.

Devmurari VP, Jivani NP (2010). Phytochemical screening and antibacterial activity of ethanolic extract of Artemisia Nilagiric*. Annals of Biological Research,* 1(1): 10-14.

Dubois M, Gilles K, Hamilton J, Rebers P and Smith F (1956). Colorimetric method For Determination of sugars and related substnces. *Anal.Chem*,28(3): 350-356.

Freeze H (1998). Disorders in Protein Glycosylation and Protein Therapy. *The Journal of Pediatrics* 133(5)543-600.

Govindarajan R, Rastogi S, Awat AKS ,Vijayakumar M, ShirwaikarA, Mehrotra S, Palpu P. (2003). Studies on the antioxidant activities of Desmodimgangeticum*. Biog Pharmaceutical, Bulletin*. 26:1424-1427.

Lowry Oliver H, Rosebrough Nira J, Farr Lewis A, Randall Rose J (1951). Protein measurement with the Folin phenol Reagent. *Journal of Biological Chemistry* 193(1):265-75.

Manjunath KP, Kulkarni GT, Patil KS (2006). Preliminary Phytochemical investigation and wounds healing activity of the leaf of Swertia chirata Buch.Huam. (Gentianaceae). *Indian Drugs*.43:535-537.

Nawaz HR, Malik A, Ali MS (2001). Trianthenol: An antifungal tetrapenoid from *Trianthema portulacastrum* (Aizoaceae). *Phytochemistry*. 56:99–102.

Saklani Sarla,Chandra Subhash, Mishra AP (2011). Evaluation of Nutritional profile, medicinal value and quantitative estimation in PyruspashiaFicus palmate and Pyracanthacrenulata. *JGTPS*, 2(3):350-354.

Shanmugam SK, Bama S, Kiruthiga N, Kumar RS, Sivakumar T, Dhanabal (2006). Investigation of analgesic activity of leaves part of the *Trianthema portulacastrum* (L) in standard experimental animal models. *Int J Green Pharm.;1:39-41.*

Singh BP, Singh RP, Jha OP (1982). Flavonoids of some Aizoaceae and Molluginaceae of Bhagalpur. *Biol Bull India*.4:157–63.

Tripathi KD (2004). *Essentials of Medical Pharmacology*. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd;.pp. 575.

Thomsen S, Handen HS, Nyman V (1991). Ribosome inhibiting proteins from invitro cultures of *Phyloceadodecandra*.*Planta. Med.* 57 232-236.

Tiwari P, Rai H, Upret DK, Trivedi IS, Shukla P (2011). Antimicrobial activity of a common Himalayan Foliose Lichen Parmotre-matinctorum (Despr. Ex Nyl. Hale*. Nature and Science*,9(9):167-171.

Vijayvergia R, Kumar J (2007). Quantification of primary metabolites of *Neriumindicum* Mill. *Asian J. Exp. Sci*. 21(1): 123-128.