

Full Length Research Paper

Preliminary Photochemical Screening and Antimicrobial activities of Plant extract of *Bryophyllum calycinum*.

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Abstract

The present study was carried out for phytochemical screening of principle bioactive compounds and antimicrobial activity in *Bryophyllum calycinum*. Phytochemical analysis revealed the presence of saponin, terpenoid, steroid, saponin, flavonoid, tannin and alkaloid. The petroleum, ether, chloroform, methanol, acetone and aqueous extracts were subjected to antimicrobial activity against bacterial strains *Staphylococcus aureus*, *Pseudomonas*, *E.coli* and *Bacillus subtilis* against anti fungal strains *A.awamori*, *A.fumigatus*, *Rhizopus oryzae*, *Trichoderma viridae* and *C.oryzae*. The antibacterial and antifungal activity was evaluated by disc-diffusion method.

Keywords: *Bryophyllum calycinum*, Antifungal activity, Antibacterial activity, Disc-diffusion method.

Introduction

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world.

India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value (Gupta et al., 2005; Sandhu and Heinrich, 2005). According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rabe Van Stoden, 2000), since they cannot afford the products of Western pharmaceutical industries (Salie et al., 1996), together with their side effects and lack of healthcare facilities (Griggs et al., 2001).

The development of bacterial resistance to presently available antibiotics has necessitated the search of new antibacterial agents. Plants and plant products are known to possess excellent antioxidant properties and play a significant role in preventing the conditions due to the excessive free radicals. (Jain et al, 2010)

The increased interest in plant derived drugs is mainly because of the wide spread belief that 'herbal medicine' is safer than costly synthetic drugs which possesses side effects. Hence, there is need to screen medicinal plants for promising biological activity. Further, there is a continuous development of resistant strains which pose them need for search and development of new drug to cure diseases (Silver, 1993).

Plant species produce valuable secondary metabolites having different types Phytochemicals or secondary metabolites usually occur in complex mixtures that differ among plant organs and stages of development (Banerjee et al., 1969). Presence of chemical and

medicinal contents in natural form, plants and herbal medicines have important position in modern medicine. They contain various secondary metabolites which work together and show wide range of antibacterial activities. Microorganisms may get mutated and become resistant to many antibiotics and so it generates a global health problem. These inspired scientists to search out new natural alternative to treat diseases (Kamboj, A and Saluja, A. K. 2009).

Bryophyllum calycinum (Synonyms: *Kalanchoe pinnatum*, *Bryophyllum pinnatum*), belongs to the family crassulaceae was commonly known as sprouting leaf (Devbhuti D, Gupta J K and Devbhuti P. 2012) . Shortly after a leaf falls to the grounds, a whole garland of new little plants develops from the notches along the leaf margin. *Bryophyllum calycinum* kurz commonly known as panfuti (Hindi), life plant, love plant, air plant (Mexican), Good luck or resurrection plant is a crassulenscent herb. They widely grow in hot and humid areas, around the dwelling places, along road sides and in abandoned farm and fields.

It is a glabrous, ornamental, crassulenscent herb, cultivated in houses and gardens. It is of about 1–1.5 m in height, with opposite, decussate, succulent, 10–20 cm long glabrous leaves (with 3–5 deeply crenulated, fleshy leaflet) with obtusely four angled stems. The lower leaves are usually simple, whereas upper ones are usually 3–7 folioate, long-petioled, petioles united by a ridge round the stem, crenatures at the extremities of the lateral nerves furnished with rooting vegetative buds. The flowers are 5cm long, reddish purple, pendent, in large spreading panicles; fruits are membranous follicles enclosed in the persistent papery calyx and corolla, seeds smooth, ellipsoid. (Kritikar K.R. and Basu B.D, 2003., Nadkarni A.K, 2005)

It grows widely and used as folk medicine in the tropical parts, Africa India, Australia and China (Yadav NP, and Dixit V.K., 2003, Lans C. A, 2006) . The plant grows all over India in hot and moist areas, especially in Bengal and Uttarakhand. The leaves and leaf juice of the plant were used traditionally as antiviral, antipyretic, antimicrobial , anti-inflammatory , antitumor , hypocholesterolemic, antioxidant, diuretic, antiulcer, styptic, antidiabetic, astringent, antiseptic, antilithic and cough suppressant (Bershtein EI., 1972, Matos FJA et al. 1984). Phytochemical studies showed that the plant contained alkaloids, phenols, steroids, terpenoids, phenolics, tannins, glycosides, carbohydrates, proteins, flavonoids, tannins, anthocyanins, glycosides, saponins, coumarins, sitosterols, quinines, carotenoids, tocopherol and lectins (Devbhuti D, et al., 2008., Pal S, et al., 1999). The petroleum ether and chloroform extracts of the powdered leaves and stems of *Bryophyllum pinnatum* showed the presence of steroids and terpenoids. The ethyl acetate extract responded positively to the tests for steroids, terpenoids, phenolics and tannins. Ethanolic extract of the leaves produced positive tests for flavonoids, steroids, terpenoids, phenolics, tannins, alkaloids and glycosides. Aqueous extract showed the presence of carbohydrates, proteins, flavonoids, phenolics, tannins and glycosides (Ojewole, 2005). Three main compounds are present in *Bryophyllum* which has their unique medicinal value. One of them is Bryophyllin A which shows strong anti-tumor activity. Others are Bersaldegenin-3-acetate and Bryophyllin C which shows insecticidal properties (Supratman et al., 2001).

All parts of *B. calycinum* are used, the leaves and the barks are the most important in the field of medicine (Chandramu et al., 2003) Extracts from the leaves of *Bryophyllum calycinum* were screened for their antimicrobial activities. Medicinal plants are rich source from which antimicrobial agents can be obtained (Kubmarawa et al., 2007). The leaves of *B. calycinum* were subjected to petroleum ether, chloroform, methanol and aqueous solvent respectively for extraction and in vitro evaluation of antimicrobial activity was done against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*.

In 1935, Chopra and Ghosh reported that the leaves and stem of *B. Calycinum* contains 0.008% of alkaloids. The Leaf of *Kalanchoepinnata* plant contains various enzymes i.e. Phosphoenol pyruvate carboxykinase, Phosphoenol pyruvate carboxylase, pyruvate orthophosphate dikinase, ribulose-1,5-biphosphate, oxygenase (Patil and Surana et. al. , 2009).

Material and method

The aerial parts of *Bryophyllum calycinum* including leaves and stems were collected around Haldwani, District Nainital, Uttarakhand, India, which is situated on piedmont grade called Bhabhar where the Mountain Rivers go underground to re-emerge in the Indo gangetic plane, it has an average elevation of 424 meters and is located at 29.22 N and 79.52 E average summer temperature ranges from 40 °C to 45 °C and winter ranges from 2 °C to 10 °C in the month of February. The collected plant materials were brought to the laboratory for the study of antimicrobial activities and phytochemical analysis.

Extraction of plant material

Fresh field grown plants were collected and washed with running tap water followed by distilled water and thoroughly dipped in 70% ethanol for removing the adhered dust particles and disinfect. After blotting, the sample was air dried in shade, ground to fine powder and stored in clean air tight containers. The powdered mixture was then soaked in different solvents ethanol, methanol, acetone, petroleum ether and chloroform for 72 hrs. After filtering the contents using Whatman No 1 filter paper, the filtrate was left at room temperature for 48 hrs to evaporate partially. Dark green, Light green, Blackish Green, Green and Yellowish green and residues were obtained. All the extracts were dried in vacuum rotary evaporator at 40 °C under reduced pressure, weighed and stored at 4 °C.

Phytochemical studies

The antimicrobial fraction obtained was subjected to various qualitative tests for the identification of constituents like alkaloids, phenols, steroids, terpenoids, phenolics, tannins, glycosides, carbohydrates, proteins, flavonoids, tannins, anthocyanins, glycosides, saponins, etc in plant extracts.

Test alkaloids

The small portions extract was stirred separately with a few drops of dil. HCl and filtered and then subjected to test for alkaloids. (Harborne, 1973).

Dragendorff's test: Extract was treated with dragendorff's reagent. Formation of orange brown precipitation which indicates the presence of alkaloids.

Mayer's test: Plant extract was treated with Mayer's reagent. Formation of cream precipitation is formed. This indicates the presence of alkaloids.

Wagner's test: Plant extract was treated with Wagner's reagent. Formation of reddish brown precipitation indicates the presence of alkaloids.

Test for tannins

In 2–3 ml of extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration indicate the presence of tannins (Trease and Evans, 1996).

Test for saponins

Few drops of olive oil was added to plant extract and vigorously shaken. Soluble emulsion is formed that indicates the presence of Saponin (Trease and Evans, 1996).

Test for reducing sugar

The plant extracts was added with the Fehling's solution (A and B) in a test tube. Colour reaction indicates the presence of reducing sugar.

Test for anthrocyanine

10% sodium hydroxide is mixed with the plant extracts. Blue colour precipitation indicates the presence of this phytochemicals.

Test for flavonoids

Alkaline reagent test: Extract was treated with minute amount of sodium hydroxide solution. Change of colour from yellow to colourless on addition of dilutes acid was observed. This indicates presence of flavonoids (Harborne, 1973).

Lead acetate test: Few drops of lead acetate solution were added with the plant extract. Yellow colour precipitation indicates the presence of flavonoids.

Test of carbohydrate

Extract was dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.

Molisch's test: Filtrate was treated with 2 drops of alcoholic α -naphthol solution in a test tube and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of violet ring at the junction indicates the presence of carbohydrates.

Benedict's test: Filtrate of plant extract was treated with Benedict's reagent and heated on water bath. Orange red precipitate indicates the presence of carbohydrate.

Test for proteins

Few ml of plant extract was added with 1 ml of 40% NaOH and 2 ml of cupric sulphate. Purple of violet colour precipitation indicates the presence of proteins.

Test for terpenoids

Thionyl chloride was added with the plant extracts. Pink colour appearance is indicating the presence of terpenoids.

Test for cardiac glycosides

Glacial acetic acid, ferric chloride and concentrated sulphuric acid were added with the plant extracts. Green colour of the mixture indicates the presence of cardiac glycosides.

Antimicrobial activity*Test microorganisms*

Antibacterial assay was carried out on *Bacillus subtilis* (gram +ve), *Staphylococcus aureus* (gram +ve), *Pseudomonas* (gram -ve), *E.coli* (gram -ve) were procured from American Type Culture Collection (ATCC), and Microbial Type Culture Collection (MTCC) Institutes. All the organisms were sub cultured and maintained on nutrient media at 37 °C.

Antifungal assay was conducted on *Aspergillus fumigatus*, *Rhizopus oryzae*, *Culbularia oryzae*, *Tricoderma virid* , *Aspergillus awamori*. Were procured from American Type culture collection (ATCC), and Microbial Type Culture Collection (MTCC) Institutes. Fungal cultures were maintained on Sabouraud dextrose agar at 30 °C.

Determination of Antibacterial and Antifungal Activity: The antibacterial and antifungal activity of all the solvent extracts of *Bryophyllum calycinum* (stems and leaves) was evaluated by disc-diffusion method. When media is solidified or set, the disc (6 mm) of whattman no 1 filter paper was soaked in crude solvent viz. methanol, ethanol, chloroform, petroleum ether, and acetone and placed carefully in the centre of Petri plates containing the solidified media. To compare the antimicrobial activity same concentration of the solvent using disc is placed in plate which acts as control to our crude solvents. Same procedure applied for the remaining Petri plates (for different solvents). The plates were incubated at 37 °C for 24 hrs for bacterial culture and for fungal culture the plates were incubated at 28 °C for 48 hrs. The plates were observed for inhibition of bacterial growth that was indicated by the clear zone around the well. The size of zone of inhibition (including well) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity. All experiments were carried out in triplicates.

Results and Discussion*Phytochemical analysis*

The preliminary phytochemical showed that the crude extract of *B. calycinum* leaf contain Reducing sugar, steroid, caumarin, phenol except saponins. While the stem of *B. calycinum* contains steroid, caumarin, phenol, saponins except reducing sugar.

Table 1. Preliminary phytochemical analysis

Phytochemical constituent	Leaf extract	Stem extract
Reducing sugar	+	-
Steroid	+	+
Phenol	+	+
Caumarin	+	+
Saponins	-	+

Antibacterial and Antifungal Activity:

The results of the antimicrobial activity tests of crude extracts are shown in table 2. It was found that twelve crude extracts of *B.calycinumat* 100mg/mL concentration exhibited various antibacterial and antifungal activity. Among the bacterial strains crude extracts of stem of *B.calycinum* were less potent then crude extracts of its leaves as they inhibited the growth of only two bacterial strains viz. *pseudomonas*, *B.subtilis* and *E. coli*. While in case of fungal strains it is concluded that leaf crude extract shows activity against *A.fumigatous* and *R.oryzae*, while the least activity was recorded by stem.

Table 2. Antimicrobial activity tests of crude extracts

Crude Solvents	Leaf Extracts				Stem Extracts		
	MT	ET	PE	CF	AC	CF	ET
Pathogenic Microorganisms							
Gram positive Bacteria							
<i>B.subtilis</i>	8	8	12	10	9	0	9
<i>S.aureus</i>	19	0	0	0	12	0	0
Gram negative Bacteria							
<i>Pseudomonas</i>	9	0	9	10	14	0	0
<i>E. coli</i>	14	8	0	0	10	8	0

Fungi							
<i>A. fumigatous</i>	10	9	8	0	0	0	12
<i>R. oryzae</i>	9	18	0	0	8	0	0
<i>A. awamori</i>	0	0	0	0	0	0	0
<i>C.oryzae</i>	0	0	0	0	0	0	0
<i>T. viridae</i>	0	0	0	0	0	0	0

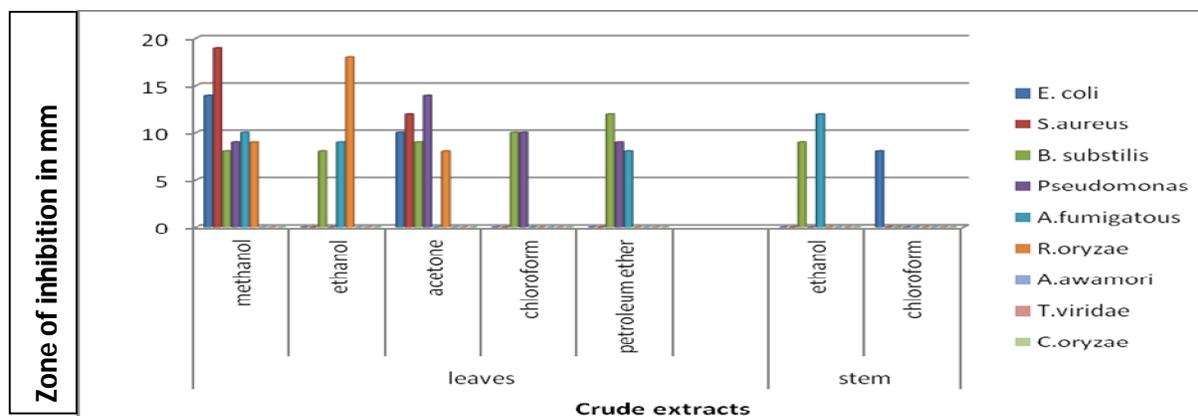


Fig. 1 Zone of inhibition in mm

Screening of antibacterial activity of leaf showed that it is potent antibacterial agent against *S.aureus*, *B.subtilis*, *Pseudomonas* and *E.coli*. Methanolic and acetone crude extract showed maximum activity followed by petroleum ether and chloroform. While least antibacterial activity was reported from ethanol but in case of *B.subtilis* it gave significant activity. Study of antibacterial activity of stem showed that it is potent antibacterial agent against *E. coli* and *B.subtilis*. No activity was reported in case of *S.aureus* and *Pseudomonas*.

In case of antifungal activity of leaf, maximum activity was found against *R. oryzae* followed by *A. fumigatous*. Further investigation revealed that methanol and ethanol crude extract was the potent antifungal agent followed by acetone and petroleum ether crude extract, while chloroform was unable to inhibit the fungal growth. Antifungal activity of stem revealed that the ethanol extracts gave activity while chloroform crude showed no activity against any of the fungal strains.

Conclusion

The present study showed that the leaf and stem extracts of *Bryophyllum calycinum*, contain many phytochemical constituents and revealed broad antimicrobial activity on the tested microorganisms. This investigation strongly suggests the possibility of this plant as an important source of antimicrobial drug development.

References

- Banerjee A. Chadha, MS . 'Phytochemical diversity of secondary metabolites', Encyclopedia of Plant & Crop Science (1969): 915-919
- Bershtein EI. Use of Kalanchoe pinnata Lam. Juice in the treatment of patients with trophic crural ulcers. Vestnik Khirurgii Imeni II Grekova; 107(3) (1972) : 116-118
- Chandramu, C., Manohar, RD., Krupadanam, DGL, Dashavantha, RV. Isolation characterization and biological activity of betulinic acid and ursolic acid from *Vitexnegundo*L. *Phytother.Res.* 17 (2003): 129–134
- Devbhuti D, Gupta JK and Devbhuti P. Studies on antitumor activity of *Bryophyllum calycinum* Salisb. against Ehrlich ascites carcinoma in Swiss albino mice. *Journal of Pharma Sci. Tech.* 2.1 (2012):31-33.
- Devbhuti D, Gupta JK, Devbhuti P and Bose A. Phytochemical and acute toxicity study on *Bryophyllum calycinum* SALISB. *Acta Poloniae Pharmaceutica-Drug Research* 65(4) (2008):501-504.
- Devbhuti D, Gupta JK, Devbhuti P and Bose A. Phytochemical and acute toxicity study on *Bryophyllum calycinum* SALISB. *Acta Poloniae Pharmaceutica-Drug Research*; 65(4) (2008):501-504
- Griggs, J K, Manandhar, N P, Towers, GHN and Taylor, R S L. The effects of storage on the biological activity of medicinal plants from Nepal. *Journal of Ethnopharmacology* 77 (2001): 247-252
- Gupta R, Lohani M, Arora S. Anti-inflammatory activity of the leaf extracts/fractions of *Bryophyllum pinnatum*; 3.1 (2010): 35 - 45
- Gupta, M P, Solis, PN, Calderon, A J, Guinneau Sinclair, F, Correa, M. Gladames, C., Guerra, C, Espinosa, A, Alvenda, GL, Robles G & Olampo, R. Medical ethnobotany of the tribes of Bocas del Toro, Panama. *Journal of Ethnopharmacology.* (2005):389-401

Harborne, J B. Phytochemical methods, First Edition, Chapman and Hall Ltd, London. (1976): 52 -55

Jain Vineet , Patel Natvarlal , Shah Dhiren , Patel Paras, Joshi Bhavesh. Antioxident and antimicrobial activities of *Bryophyllum calycinum* leaf. (2010):393-405

Kamboj A, Saluja AK. *Bryophyllum pinnatum* (Lam.) Kurz.: Phytochemical and pharmacological profile : A review. Phcog Rev (serial online) (2009) [cited 2016 Jan 27];3:364-74

Kamboj and Saluja A K. "Bryophyllum pinnatum (Lam.) Kurz. Phytochemical and pharmacological profile: A review," *Pharmacognosy Review*, vol. 3.3 (2009) 64-74.

Kritikar KR. and Basu BD. Indian medicinal plants Illustrations. Volume-5, Second edition 2003, published by oriental enterprises, (2003):1394-96

Kubmarawa, D, Ajoku, GA, Enweram N M. and Okorie D A. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *Afric.J. Biotechnol.*, 64 (2007): 1690-1696

Lans C A. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *Journal of Ethnobiology and Ethnomedicine* (2006): 45

Matos FJA. Plantas da Medicina Popular do Ceara Seleccionadas pela Maior Frequencia de Seu Uso, VIII Simposio de Plantas Mediciniais do, Brazil, (1984): 24

Nadkarni AK. Indian Materia Medica, Volume-1, Third edition 2005, Published by Popular Prakashan, Bombay. (2005):1402.

Ojewole JAO. Antinociceptive, anti-inflammatory and antidiabetic effects of *Bryophyllum pinnatum* (Crassulaceae) leaf aqueous extract. *J. Ethnopharmacol.* 99(1) (2005) :13-19.

Pal S, Sen T, and Nag Chaudhari AK. Neuropsychopharmacological profile of the methanolic fraction of *Bryophyllum Pinnatum* leaf extract. *Journal of Pharmacy and Pharmacology* ; 51 (1999) : 313-318

Patil R, Bhargava K, Patel P, Singh K, Surana J. Diuretic and anti-urolithiatic activity of Hydroalcoholic extract of leaves of *Kalanchoepinnata*. *J. Pharm. Res.*; 7(2) (2009) : 87-91

Rabe, T. and Van stoden, J. Isolation of an antimicrobial sesquiterp enoid from *Warbugie salutaris*. *Journal of Pharmacology.* 93 (2000):171-174

Salie F, Eagles PFK, Lens HMJ . Preliminary antimicrobial screening of four South African Asteraceae species. *J. Ethnopharmacol.* 52(1) (1996): 27-33

Sandhu D S , Heinrich M., The use of health food spices and other botanical in the sikh community in London phytotherapy Res.19 (2005): 633- 42

Silver L L. Discovery and development of new antibiotics: the problem of antibiotic resistance. *Antimicrob.Agents.Chemother.* 37 (1993): 377-383

Supratman U, Fujita T, Akiyama K, Hayashi H, Murakami A, Sakai H, Koshimizu K, Ohigashi H. Anti-tumor promoting activity of bufadienolides from *Kalanchoepinnata* and *K. daigremontiana* x *tubiflora*. *Bioscience Biotechnology Biochemistry*; 65(4) (2001): 947-949

Trease GE, Evans, W C. A textbook of pharmacognosy. 12thEdn., Bailliere, Tindall. London (1989): 388, 480, 502, 535, 546

Yadav NP, Dixit VK. Hepatoprotective activity of leaves of *Kalanchoe pinnata* Pers, *Journal of Ethnopharmacology* (2003); 86:197–202