WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 1, 849-858. Research Article ISSN 2277– 7105

PHYTOCHEMICAL AND ANTIFUNGAL ACTIVITY OF CLEOME RUTIDOSPERMA PLANT EXTRACTS

Mullai Nila K.[1] and Karthikeyan J.*[1]

Department of Zoology, Presidency College (Autonomous), Chennai- 600 005.

Article Received on
06 Nov. 2017,

Revised on 28 Nov. 2017,
Accepted on 18 Dec. 2017

DOI: 10.20959/wjpr20181-10509

***Corresponding Author**
Karthikeyan J.
Department of Zoology, Presidency College (Autonomous), Chennai- 600 005.

ABSTRACT

Antifungal activity of *Cleome rutidosperma* methanol and aqueous leaf extract was studied against *Aspergillus niger*, *Mucor indicus* and *Rhizopus microsporus*. The preliminary phytochemical studies were performed and the presence of alkaloids, flavonoids, steroids, cardiac glycosides, protein, tannin, phenols and carbohydrates in the methanol extract and phenol, saponins and protein were found in aqueous extract. The antifungal activity was performed using disc diffusion method at various concentration of 25mg/ml, 50mg/ml and 100mg/ml of extracts and the zone of inhibition was measured after 48h of incubation. The results observed in the methanolic leaf extract are comparatively higher with reference to positive control Clotrimazole. In aqueous extract minimal or no zone of inhibition was observed. Antifungal activity of the methanolic extract may be due to the presence of phenolic compounds. Hence, the separation and purification of fungicidal compounds from *Cleome rutidosperma* may throw light on the identification of potential antifungal agent.

KEYWORDS: *Cleome rutidosperma*, antifungal activity, *Aspergillus niger*, *Mucor indicus*, *Rhizopus microsporus*, phenolic compounds.

INTRODUCTION

Many individuals depend on natural plants as a source of treatment for different diseases till date. Most of the medicines were derived from natural sources.[1] Medicinal plants play a major role in the reduction of mortality by curing many infectious diseases.[2] Screening of plants for their phytochemical composition and biological activities is one of the major streams of research of international concern.[3] Unsystematic applications of synthetic drugs result in resistance among microbial pathogens responsible for serious infections.[4] It leads to

the quench of searching novel drugs with resistance combating potential.[5] Medicinal plants act as a reservoir of diverse secondary metabolites with tremendous medicinal value, which can be exploited for the isolation and identification of new substance with antimicrobial activity.[6] Infections caused by microorganisms are alarming around the world and becomes a major threat to human welfare.[7] Minimal research has been carried out on the antifungal activity of medicinal plants.[8]

Fungi are ubiquitous in nature and the infection caused by the fungi turned out to be more incessant.[9,10] It is very difficult to control the growth of fungi due to their ability to metabolize many substances.[11] In humans, fungal infections vary from superficial to deeply invasive nature and the treatment of mycoses is far behind when compared to bacterial chemotherapy. Research has been carried out to identify the natural fungicidal compounds to alternate the synthetic drugs available in the market. Antifungal agents obtained from the medicinal plants are more preferred due to their nontoxic and environmental friendly nature.

India is one of the countries with a large resource of medicinal plants which are therapeutically valuable.[12] *Cleome rutidosperma*, commonly known as "Fringed Spider Flower" of family Cleomaceae (Figure 1), was a tiny herb that grows up to 70 cm height with trifoliate leaves bearing little violet-blue flowers. This plant is native to West Africa and becomes naturalized in tropical and temperate regions of different parts of the World.[13]

Fig. 1: *Cleome rutidosperma* Plant.

The *Cleome rutidosperma* has been studied for its medicinal properties such as antibacterial, antioxidant,[14] anti-diabetic,[15] anti-inflammatory and anti-hyperglycemic activity.[16] Hence, the present study is intended to analyze the antifungal activity of different leaf extracts of *Cleome rutidosperma* against disease-causing fungal organisms.

MATERIALS AND METHODS

Collection of Plant

The whole plant of *Cleome rutidosperma* was collected from Koyambedu, Chennai, Tamil Nadu India. The plant was identified taxonomically and authenticated at the P. G Research Department of Plant Biology and Biotechnology, Presidency College, Chennai, Tamil Nadu, India. The disease-free leaves were separated and washed totally with tap water followed by rinsing with double distilled water and shade dried at temperatures between 28ºC and 30ºC. The dried leaves were fine powdered by using a mechanical blender and the powder was stored in the desiccator.

Extraction procedure

Aqueous extraction process

Hundred grams of *Cleome rutidosperma* leaf powder was placed in a stripper container with 1 lit of distilled water and allowed to stand at room temperature for a period of 72 h with continuous stirring agitation. The mixture has been strained and the filtrate is allowed to concentrate at 40 ºC in a water bath.

Extraction of methanol

The dried powder (25g) of *Cleome rutidosperma* was packed in a thimble made up of filter paper placed in the chamber of Soxhlet device with 250 ml of methanol as solvent and the process is continuous for a period of 8 h till the complete extraction of phytocompounds. The concentrated extracts were refrigerated.

Preliminary Phytochemical Screening

Preliminary chemical analysis was performed to analyze the various phyto-components of *Cleome rutidosperma* leaf.[17,18]

Test for Alkaloids

Five ml of 1% Hydrochloric acid and 100 mg of each extract was dissolved separately and filtered. The filtrate was treated with few drops of Dragendorff's reagent. The appearance of orange-red precipitate shows the presence of alkaloids.

Test for Flavonoids

The plant extract was dissolved in methanol by mild heating and few fragments of magnesium ribbon were added, followed by addition of few drops of concentrated

hydrochloric acid. The colour changes from orange, pink and red to purple showing the presence of flavonoids.

Test for Cardiac Glycosides

Keller- Killani test

Three ml of each plant extract was added to 2 ml of glacial acetic acid and one drop of 1% ferric chloride solution and 1 ml of concentrated sulphuric acid were added. A brown ring formed at the interface indicates the presence of cardiac glycosides.

Test for Steroids

Hundred mg of extract was dissolved in equal volume of acetic anhydride and chloroform and cooled at 0ºC for few minutes. By adding few drops of concentrated sulphuric acid, reddish brown or violet-brown ring was formed which shows the presence of steroids.

Test for Protein

Two ml of each extract was treated with few drops of 1% Ninhydrin solution. On mild heating in the water bath it produced blue colour confirms the presence of protein.

Test for Tannins

Two hundred mg of each extract was dissolved in 10 ml of distilled water then filtered. Few drops of 1% alcoholic ferric chloride solution were added to 2 ml of each extract taken in separate test tubes. The formation of blue-green precipitate confirms the presence of tannins.

Test for Carbohydrates

Five ml of extract was treated with equal volume of 1 ml Fehling A and 1 ml Fehling B solutions, boiled for 1 min separately. The mixtures were boiled for 5-10 minutes in water bath. Reddish brown color was obtained because of the formation of cuprous oxide which indicated the presence of reducing sugar.

Test for Phenols

One ml of each extract was added with 3-4 drops of 1% ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Test for Saponins

One gram of each extract was vigorously shaken with 3 to 5 ml of distilled water. The foam persists for 10 minutes confirming the presence of saponins.

Antifungal Bioassay

The antifungal activity of aqueous and methanol leaf extracts of *Cleome rutidosperma* was tested against *Aspergillus niger*, *Mucor indicus* and *Rhizopus microsporus*. Fungal cultures were collected from the Department of Microbiology, Presidency College, Chennai, Tamil Nadu, India. The culture was sub cultured on Nutrient agar slants and stored at 4ºC till use. The susceptibility of the tested organisms to crude methanol and aqueous extracts of *Cleome rutidosperma* was tested by disc diffusion method.[19] The Sabouraud Dextrose Agar (Hi-media, Mumbai) plates were inoculated with each fungal culture. The discs (6mm in diameter) were impregnated with 25mg/ml, 50mg/ml and 100mg/ml concentrations of the each extract and were placed on the plates. Fungicide Clotrimazole (20µg/ml) was used as positive control. The activity was determined after 48h of incubation at 28ºC. The diameters of the clear zone of inhibition were measured in millimeter (mm).

Statistical evaluation

The values of each sample were expressed as Mean ± SD of three replicates.

RESULT

The antifungal efficacy of crude leaf extract of *Cleome rutidosperma* was tested against *Aspergillus niger*, *Mucor indicus* and *Rhizopus microsporus* and the activity was measured by zone of inhibition, Clotrimazole (20µg/ml) has been used as positive control. A dose-dependent antifungal activity was observed in the tested organisms. In methanol extract the zone of inhibition (mm) varies from 10.25±0.11 to 11.25±0.11 with reference to *Mucor indicus*, 9.00±1.80 to 11.50±2.30 with reference to *Aspergillus niger* and 11.75±0.11 to 13.25±0.11 with reference to *Rhizopus microsporus*, where the zone of inhibition with 100 mg concentration was significantly higher when compared to positive control against the organisms tested in the present study (Table 1, Figure 2). However, the fungicidal efficacy of the aqueous extract was very meager and there was no significant antifungal activity was observed in *Aspergillus niger* and *Rhizopus microsporus*, no zone of inhibition was also observed with reference to *Mucor indicus* (Table 2, Figure 3). From the above findings it is evident that the crude methanol leaf extract contains the potential fungicidal compounds when compared to aqueous extract. The results presented in Table 3 shows the presence of various bioactive compounds in the aqueous and methanol extract. Presence of major bioactive compounds such as alkaloids, flavonoids, cardiac glycosides, phenols, tannins was

reported in the methanolic leaf extract, while the presence of phenol and saponins were evident in aqueous extract.

Table 1: Antifungal activity of methanol leaf extract of *Cleome rutidosperma.*

Microbes	ZOI (mm)			
	25 mg/ml	50mg/ml	100mg/ml	Clotrimazole 20µg/ml
Aspergillus niger	9.00±1.80	10.67±2.14	11.50±2.30	6.50±1.30
Mucor indicus	10.25±0.11	11.17±0.11	11.25±0.11	10.25±0.11
Rhizopus microsporus	11.75±0.11	13.17±0.11	13.25±0.11	11.25±0.11

ZOI- Zone of Inhibition; mm- millimeter

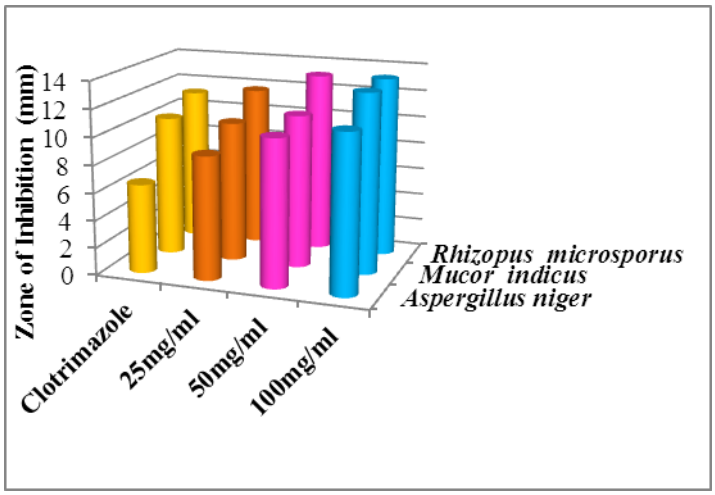

Fig. 2: Antifungal activity of methanol leaf extract of *Cleome rutidosperma* against *Aspergillus niger, Mucor indicus, Rhizopus microsporus.*

Table 2: Antifungal activity of aqueous extract of *Cleome rutidosperma.*

Microbes	ZOI (mm)			
	25 mg/ml	50mg/ml	100mg/ml	Clotrimazole 20µg/ml
Aspergillus niger	0.00±0.00	6.08±1.24	6.17±1.23	6.25±1.26
Mucor indicus	0.00±0.00	0.00±0.00	0.00±0.00	9.00±0.11
Rhizopus microsporus	7.33±0.17	8.25±0.18	8.50±0.11	10.05±0.11

ZOI- Zone of Inhibition; mm- millimeter.

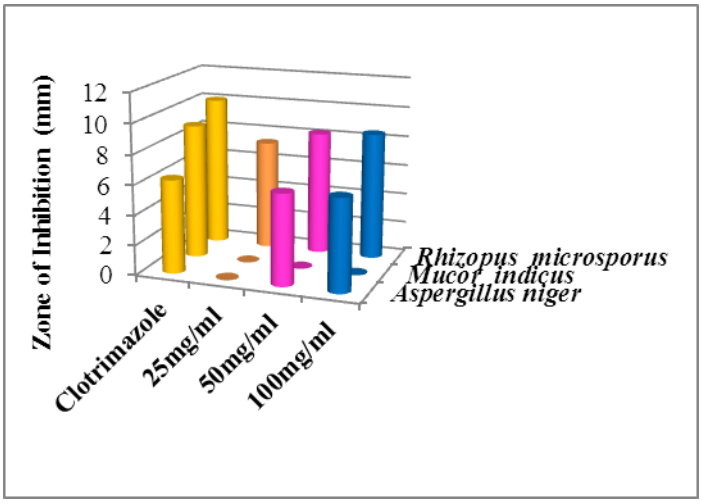

Fig. 3: Antifungal activity of aqueous leaf extracts of *Cleome rutidosperma* against *Aspergillus niger, Mucor indicus, Rhizopus microsporus.*

Table 3: Phytochemical analysis of *Cleome rutidosperma*

Phytoconstituents	Methanol	Aqueous
Alkaloids	++	-
Flavonoids	+++	-
Cardiac glycosides	+	-
Phenols	+	+
Tannin	+	-
Steroids	++	-
Carbohydrates	+	-
Protein	+	+
Saponin	-	+

(+++) Highly present (++) moderately present (-) absent

DISCUSSION

The phytocompounds isolated from the medicinal plants has significant therapeutic potential against infections caused by bacteria, fungi and virus. The broad spectrum antifungal activity of the plant extracts are due to the presence of various chemical constituents which can act individually or in combinations with other phytoconstituents.[20] The methanolic extract of *P. karka* and *V. ziza* shows antifungal activity against *Candida albicans* and *Rhizopus nigricans.*[21] The plant-derived compounds such as hydroquinones, flavonoids, alkaloids were termed as potential antifungal agents.[22] The preliminary analysis of crude extract shows the presence of various secondary metabolites. A maximum amount of flavonoids and phenols were observed in the present study. The flavonoids were considered as larger groups of phenolic compounds. Phenolic compound are the most important group of phytocompounds with more than 10,000 individual compounds.[23] The antifungal activity of

fruit residues of Brazilian Savanna species was attributed by the presence of phenolic compounds.[24]

The present study reveals the potential fungicidal activity of the crude methanolic leaf extract of *Cleome rutidosperma* against *Aspergillus niger*, *Mucor indicus* and *Rhizopus microsporus*. The antifungal properties of the extracts may be due to the presence of high amount of phenolic compounds such as flavonoids in the extracts. The possible mechanism of action of the phenolic compounds may be due to the impairment of enzymatic process or by destroying the permeability barrier of the cell membrane.[25] The phenolic compounds may act on the denaturation of enzymes responsible for spore germination.[26] The present study also suggests that the phenolic compounds are responsible for the potential antifungal activity of the crude methanol extract. Moreover it is also evident that the aqueous extract without the presence of phenolic compounds shows a very minimal or no antifungal activity against the organisms tested.

CONCLUSION

In conclusion, the methanolic leaf extract possesses potential compound with antifungal activity. The isolation and characterization of the phenolic compounds will paves the way for the development of new therapeutic agents against the fungal diseases.

REFERENCES

1. Ntsoelinyane PH, Mashele SS, Manduna IT. The anticancer, antioxidant and phytochemical screening of *Philenoptera violacea* and *Xanthocercis zambesiaca* leaf, flower and twig extracts. *Int J Pharm Res*, 2014; 4(3): 100-05.
2. Bulbul IJ, Jahan N. Study on antioxidant and antimicrobial activities of methanolic leaf extract of *Glycosmis pentaphylla* against various microbial strains. *J Pharm Phytochem*, 2016; 5(4): 53-7.
3. Osbourn AE, Lanzotti V. Plant-derived Natural Products: Synthesis, Function, and Application. Springer, New York, 2009; DOI 10.1007/978-0-387-85498-4.
4. Bax R, Mullan N, Verhoef J. The millennium bugs—the need for and development of new antibacterials. *Int J Antimicrob Agents*, 2000; 16: 51–9.
5. Muhaidat R, Al-Qudah MA, Samir O, Jacob JH, Hussein E, Al-Tarawneh IN, Bsoul E, Abu Orabi ST. Phytochemical investigation and *in vitro* antibacterial activity of essential

oils from *Cleome droserifolia* (Forssk.) Delile and *C. trinervia* Fresen. (Cleomaceae). *South Afr J Bot*, 2015; 99: 21-8.

6. Pan L, Esperanza J, Carcache DB, Kinghorn, AD. Plant-derived natural products as leads for drug discovery. In: Osbourn, A.E., Lanzotti, V. (Eds.), Plant derived Natural Products: Synthesis, Function, and Application. Springer, New York, 2009; 547–68.
7. Akinpelu DA, Abioye EO, Aiyegoro OA, Akinpelu OF, Okoh AI. Evaluation of antibacterial and antifungal properties of *Alchornea laxiflora* (Benth.) Pax. & Hoffman. *Evidence Based Complement Altern Med*, 2015; 1-6.
8. Webster D, Taschereau P, Belland RJ, Sand C, Rennie RP. Antifungal activity of medicinal plant extracts; preliminary screening studies. *J Ethnopharmacol*, 2008; 115(1): 140-46.
9. Walsh TJ, Groll AH. Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty – first century. *Transpl Infect Dis*, 1999; 1(4): 247-61.
10. Fleming RV, Walsh TJ, Anaissie EJ. Emerging and less common fungal pathogens. *Infect Dis Clin North Am*, 2002; 16: 915-33.
11. Quiroga EN, Sampietro AR, Vattuone MA. Screening antifungal activities of selected medicinal plants. *J Ethnopharmacol*, 2001; 74(1): 89-96.
12. Sharma S, Thokchom R. A review on endangered medicinal plants of India and their conservation. *J Crop Weed*, 2014; 10(2): 205-18.
13. Waterhouse B, Mitchell A. Northern Australia Quarantine Strategy Weeds Target List. 2nd ed., Canberra; AQIS Miscellaneous Publication, 1998; 29.
14. Chakraborty AK, Charde MS, Roy H, Bhanja S, Behera M. Comparative study of antioxidant activity between ethanolic and aqueous extract of *Cleome rutidosperma*. *Int J Pharm Sci Res*, 2010; 1(11): 112- 16.
15. Okoro IO, Umar IA, Atawodi SE, Anigo KM**.** *In vitro* and *in vivo* antihyperglycemic effect of active fraction of C*leome rutidosperma* DC. *Int J Pharm Pharm Sci*, 2015; 7(1): 289-95.
16. Ansari P, Debnath M, Ahmad MF, Azam S, Akther S, Mustakim GM, Naquib MH, Sarwar S. Evaluation of Antinociceptive activity of methanol extract from *Cleome rutidopserma* in mice. *Chinese Herbal Medi*, 2016; 8(3): 273-79.
17. Harborne IB. Phytochemical methods: A guide to modern techniques of plant analysis. 2nd ed., New York; Chapman and Hall, 1973; 88-185.

18. Trease GE, Evans WC. Phytochemicals. In: Pharmacognosy. London; Saunders Publishers, 2002; 528-33.
19. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol,* 1966; 45: 493-96.
20. Khuntia A, Mohanty SK. Antifungal activity of *Cleome rutidosperma* aerial parts. *Res J Pharm Tech*, 2011; 4(7): 1103-105.
21. Soni A, Dahiya P. Screening of phytochemicals and antimicrobial potential of extracts of *Vetiver zizanoides* and *Phragmites karka* against clinical isolates. *Int J Appl Pharm*, 2015; 7(1): 22-24.
22. Galven IJ, Mir-Rashad N, Jessulat M, Atanya M, Golshani A, Durst T, Petit P, Amiquet VT, Boekhout T, Summerbell R, Cruz I, Arnason J, T Smith ML. Antifungal and antioxidant activities of the phytomedicine pipsissewa, *Chimaphila umbellate*. *Phytochemistry*, 2008; 69(3): 738-46.
23. Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, Dabur R. Natural products – antifungal agents derived from plants. *J Asian Nat Prod Res*, 2009; 11(7): 621-38.
24. Breda CA, Gasperini AM, Garcia VL, Monteiro KM, Bataglion GA, Eberlin MN, Duart MC. Phytochemical analysis and antifungal activity of extracts from leaves and fruit residues of Brazilian savanna plants aiming its use as safe fungicides. *Nat Prod Bioprospect*, 2016; 6(4): 195-204.
25. Cutter CN. Antimicrobial effect of herb extracts against *Escherichia coli*- 0157: H7, *Listeria monocytogenes*, *Salmonella typhimurium* associated with beef. *J Food Prot*, 2000; 63(5): 601- 607.
26. Nychas GJE. In: New methods of food preservation. Gould GW. Editor., New York: Blackie Academic and Professional, 1995; 58-69.