International Journal of Pharmacological Research
ISSN: 2277-3312
www.ssjournals.com
Journal DOI:10.7439/ijpr

Evaluation of antimicrobial activity of ethanolic extract of *Dactyloctenium aegyptium*

Veeresh Kumar[*1], Rauf Fathima Banu[1], Sulthana Begum[1], M. Satish Kumar[2], T. Mangilal[3]

[1]*JPNES Group of Institutions, Faculty of Pharmacy, Mahabubnagar, India*
[2]*Geethanjali College of Pharmacy, Cheeryal, Rangareddy District, India*
[3]*Department of Pharmacy, UCT, Osmania University, Hyderabad, India*

Corresponding author*

Veeresh Kumar P.
JPNES Group of Institutions,
Faculty of Pharmacy,
Mahabubnagar-509001, Telangana, India
E-mail: veeresh.pmahendra@gmail.com

Abstract

Dactyloctenium aegyptium is an Indian medicinal plant to provide fuel, fodder and stabilizes soil in natural woodland and plantations. *Dactyloctenium aegyptium* is known for its antimicrobial activity, but the antifungal effects of Ethanolic extract on growth of *Aspergillus niger* have been observed. The extract showed a favorable antifungal activity against *Aspergillus niger*. Ethanolic extract of *Dactyloctenium aegyptium* were examined for their phytochemical compounds and antimicrobial potential against three standard bacteria (*Escherichia coli, Klebsiella Pneumonia, Staphylococci*) and one standard fungus (*Aspergillus niger*).The phytochemical analysis showed the presence of some active principle which correlates with the antifungal activity of ethanolic extract of *Dactyloctenium aegyptium*. The ethanolic extract of *Dactyloctenium aegyptium* shows the maximum antifungal activity compared to Griseoflavin.

Keywords: *Dactyloctenium aegyptium, Aspergillus niger, Escherichia coli, Klebsiella Pneumonia, Staphylococci*, antifungal activity, Griseoflavin

1. Introduction

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern [1]. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidermis of human immunodeficiency virus (HIV) infections[2][3]. This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants [4]. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections [5].

The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens [6]. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and an urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [7]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [8]. The increasing failure of chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential antimicrobial activity [9][10].

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties [11]. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [12]. Thus, it is anticipated that photochemical with adequate antibacterial efficacy will be used for the treatment of bacterial infections [13]. Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments [14].

Plants were the mainstay of medicine and credited with mystical and almost supernatural powers of healing. The

practice of herbal medicine dates back to the very earliest periods of known history. There is evidence of the herb have been used in treatment of diseases and for revitalizing body systems in almost all ancient civilizations. Medicinal plants were existing even before human beings made their appearance on the earth. It is therefore often said that where ever we are born, we have around us herbs, shrubs and plants which are useful for us [15].

The use of plants, plant extracts or plant derived pure chemicals to treat disease is therapeutic modality, which has stood the test of time. Indeed, many pharmacological classes of drugs, including a natural product prototype. Aspirin, Atropine, Ephedrine, Digoxin, Morphine, Quinine, Reserpine and Tubocurarine are a few examples of drugs, which were originally discovered through the study of traditional cures and folk knowledge of indigenous people. There is a revived interest in herbal products (botanicals) at a goal level and conventional medicine is now beginning to accept the use of botanicals once they are scientifically validated. Isphagula, Garlic, Ginseng, Ginger, Ginkgo, St. John's Wort and a Saw palmetto are a few examples of botanicals which are gaining popularity amongst modern physicians and this trend is likely to continue, partly due to the high cost involved in the development of patentable chemical drugs. There is growing evidence to show that medicinal plants contain synergistic and/or side-effects neutralizing combinations. Ethnopharmacology has already played an important role in the development of conventional medicine and is likely to play more significant role in the years to come [16].

During the later part of this century the practice of Herbalism has become main stream throughout the world. This is due in part to the recognition of the value of traditional medicinal systems, particularly of Asian origin, and the identification of medicinal plants from indigenous pharmacopoeias that have been shown to have significant healing power, either in their natural state or as the source of new pharmaceuticals. Generally, these formulations are considered moderately in efficacy and thus less toxic than most pharmaceutical agents. In the western world, in particular, the developing concept that 'natural' is better than 'chemical' or 'synthetic' has led to the evaluation of Neo-western Herbalism that is the basis of an ever expanding industry. In the U.S, often used as food or food supplements, known as neutraceuticals, these formulations are readily available for those that wish to self medicate [17].

A team work amongst Ethno botanist, Ethno pharmacologist, physicians and phytochemist is essential for the fruitful outcome on medicinal plant research, while the Ethno pharmacologist has greater role to play in the rationalization of the combination of activities, the phytochemist's role will slightly shift towards standardization of botanicals[18].

The main aim of the present study is "*Dactyloctenium aegyptium* has been documented to possess, worm infection respectively, but the effect *of Dactyloctenium aegyptium* for Antimicrobial activity is still not reported. Hence it was thought worthwhile to screen extract of *Dactyloctenium aegyptium* as an Antimicrobial activity. So the aim of the present study will be Evaluation of Antimicrobial activity of Ethanolic extract of Dactyloctenium aegyptium.

2. Materials and Methods

2.1 Collection of plant materials:

Dactyloctenium aegyptium vine was collected from the Botanical Garden attached to University College of Science, Osmania University, Hyderabad, Telangana, India and authenticated at the Department of botany of the same University.

2.2 Preparation of plant extracts

The collected plant materials were washed, sliced, and completely dried in a hot air oven at 60oC. The dried materials were ground and aerated in 95% ethanol for three days and filtered. The marc was remacerated in 95% ethanol for another three days and filtered. The two sets of the filtrate were pooled and evaporated to give crude extract, which was dissolved in mixed solvents of methanol and water (9:1). The dissolved crude extract was re-extracted with an equal volume of hexane, dichrolomethane, and butanol in succession at least three to four times for each solvent. The extracts obtained from each solvent were combined and concentrated to dryness under reduced pressure [19].

2.3 Phytochemical Analysis

Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by Rizk and Bashir. The plant extracts were screened for the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids [20]. *Dactyloctenium aegyptium* was evaporated to residue and diluted HCl was added into it. After shaking, the extract was filtered and filtrate tests were performed for the detection of following constituents.

2.4 Identification test

The test were carried out to find the presence of the active chemical constituents such as glycosides, Phenolics, Alkaloids, Tannins, Flavonoids, Saponins and Steroids by the following procedure.

2.4.1 Test for Alkaloids

2ml filtrate of the plant extract was mixed with 2ml of HCl and about 6 drops of Mayor's reagents. A creamish or

pale yellow precipitate indicates the presence of alkaloids[21].

2.4.2 Test for steroids

One ml of the extract was dissolved in 2 ml of acetic anhydride which is then added to 2 ml of H_2SO_4. The color changes from violet to blue or green in some samples, indicating the presence of steroids [22].

2.4.3 Test for tannins

One ml of the extract was treated with a few drops of 1% ferric chloride solution and observed for brownish green or a blue-black coloration, confirming presence of tannins [23].

2.4.5 Test for flavonoids

Four ml of filtrate was added to 5-6 drops of conc. HCl and1.5 ml of methanol solution. Pink-tomato red color indicates the presence of flavonoids[24].

2.4.6 Test for glycosides

Two ml of extract is mixed with 2 ml chloroform. Then 2ml acetic anhydride and 2 drops of conc. H_2SO_4 was added from the side of the test tube. First red, then blue and finally green colour appears indicating presence of glycosides [25].

2.4.7 Test for Phenolics

Two ml of ethanol was added to the test solution and a few drops of ferric chloride solution. Blue coloration indicates the presence of Phenolics[26].

2.4.8 Test for Saponins

2ml of distilled water was added to 2ml of the test solution and shaken well and observed for frothing. Presence of various constituents in different solvent was determined [27].

2.5 Study of antimicrobial activity

The selected compounds prepared in the course of the present investigation are screened for antibacterial activity against the following bacteria's. *Staphylococus aureus* (Gram +ve) (MTCC 737),*Escherichia coli* (Gram -ve) (MTCC 1687),*Strepto cocci* (Gram +ve) (MTCC 3086),*Pseudomonas* (Gram -ve) (MTCC 1035) and antifungal activity against following fungi,*Aspergillus niger* (MTCC 2638).

2.5.1 Antibacterial Activity

The antibacterial activities are performed by cup-plate method (diffusion technique). The fresh culture of bacteria is obtained by inoculating bacteria into peptone water liquid media and incubated at 37 ± 2^0C for 18 – 24 hours. This culture mixed with nutrient agar media (20%) and poured into petri dishes by following aseptic techniques. After solidification of the media five bores are made at equal distance by using sterile steel cork borer (8 mm diameter). Into these cups different concentrations of standard drugs and synthesized compounds are introduced. Dimethyl formamide was used as a control. After the introduction of standard drugs and synthesized compounds, the plates were placed in a refrigerator at 8^0 -10^0C for proper diffusion of drugs into the media. After two hours of cold incubation, the petri plates are transferred into incubator and maintained at 37^0±2^0C for 18-24 hours. After the incubation period, the petri plates were observed for the zone of inhibition by using vernier scale. The results evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drugs. The results are the mean value of zone of inhibition measured in millimeter of two sets. The standard drugs and synthesized compounds were dissolved in a minimum quantity of N N' dimethyl formamide (DMF) and adjusted and the volume with distilled water to get 50mg/ml and 100mg/ml concentrations. The procaine penicillin, streptomycin used against *Staphylococcus aureus, Escherichia coli*, and Cetazolin Sodium, Sporafloxin used against *Pseudomonas, strepto cocci*[28].

Table 1: Preparation of subculture media

Ingredients	Quantity
Peptone	10 gm
Beef extract	10 gm
Sodium chloride	5 gm
Distilled water	Q. S. 1000 ml

Table 2 Preparation of assay medium

Ingredients	Quantity
Nutrient agar media	28.0gms
Agar and agar	4.0gms
Distilled water	Q. S. 1000 ml

2.5.2 Antifungal activity

The synthesized compounds are screened against two selected fungal strain *Aspergillus niger* by using diffusion method. The 48 hours old fungal culture inoculated into a nutrient broth by following aseptic techniques and incubated for

48 hours at $37^0\pm 2^0$C in an incubator. This culture mixed with Potato-dextrose agar media (20%) and poured into petri plates. After solidification five bores are made at equal distance by using sterile steel cork borer (8 mm in diameter). Into these cups different concentrations of standard drug and synthesized compounds along with control DMF (N N' Dimethyl formamide) introduced. After the introduction of standard drug and compounds, these plates are placed in a refrigerator at 8^0-10^0C for two hours for proper diffusion of the drugs. After 2 hours of cold incubation, the petri plates are transferred to an incubator and maintained at $37^0\pm 2^0$C for 24-36 hours. After the incubation period, the plates were observed in the zone of inhibition by using vernier scale. The results evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drug. The results are the mean value of zone of inhibition measured in millimeter of two sets. The results are tabulated in the Table No. 7. The standard drug and synthesized compounds were dissolved in a minimum quantity of DMF and adjusted, to make up the volume with distilled water to get 50µg/ml and 100µg/ml concentrations. The Griseoflavin used as a standard drug[29].

Table 3: Composition of nutrient broth

Ingredients	Quantity
Peptone	10.0 gm
Yeast extract	6.0 gm
Potassium dihydrogen phosphate	3.0 gm
Sodium chloride	5.0 gm
Glucose (anhydrous)	10.0 gm
Distilled water	Q. S. 1000.0 ml

Table 4: Potato – dextrose agar medium

Ingredients	Quantity
Potato Dextrose media	39.0 gm
Agar agar	4.0 gm
Distilled water	Q. S. 1000.0 ml

3. Results and Discussion

A review of the chemistry, the pharmacology and therapeutic potency of different dactolyniem species has been reported. *Dactyloctenium aegyptium* has particularly shown some interesting biological activities related to its worldwide uses in traditional medicine.

Preliminary phytochemical analysis revealed the presence of alkaloids and saponins. The other secondary metabolites like tannins, flavonoids, steroids etc. were present in trace amounts in some of the plants and results is shown in table 4.

Table 4: Phytochemical Tests

S. No.	Chemical Test	Aqueous Extract
1.	Alkaloids	-
	Mayer's test	+++
	Dragondroff's test	+++
	Wagner's Test	+++
	Hager's Test	+++
2.	Steroids	+++
3.	Tannins	++
4.	Fixed oils	++
5.	Tannins	++
6.	Phenols	-
a).	Ferric chloride	++
b).	Lead acetate	++
c).	Gelatin solution	++
7.	Saponins	-
a).	Foam test	+
9.	Flavoniods	-
a).	Aqueous NaOH Test	++
b).	Conc. H_2SO_4 Test	++
c).	Schinodo's test	++

+++ Indicates High, ++ Indicates Intermediate, + Indicates Low, - Indicates Absent.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials.

Continued further exploration of plant- derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy. However, the present study of in vitro antimicrobial evaluation of some plant forms a primary platform for further phytochemical and pharmacological studies. Ethanolic extract of *Dactyloctenium aegyptium* showed zone of inhibition. There is a dose dependent increase in zone of inhibition. *Dactyloctenium aegyptium* (200mg/kg) inhibits more colonies than that of *Dactyloctenium aegyptiumaegyptium* (100mg/kg). The ethanolic extract of *Dactyloctenium aegyptium* was found to be effective in the dose of 100mg/kg and 200mg/kg respectively, and results are shown in table 5 and figure 1.

Table 5: Antibacterial activity

Sl. No	Name of the compounds	Mean zone of inhibition (in mm)*							
		Staphylococcus aureus		*Escherichia coli*		*Enterococci faecelis*		*Pseudomonas auruginosa*	
		100µg	200µg	100µg	200µg	100µg	200µg	100µg	200µg
1	Procaine penicillin	18	20	-	-	-	-	-	-
2	Streptomycin	-	-	28	39	-	-	-	-
3	Sporofloxin	-	-	-	-	33	40	-	-
4	Ciprofloxacin	-	-	-	-	-	-	18	24
5	*Dactyloctenium aegyptium*	-	-	20(0.71)	29(0.74)	-	-	-	-

The ethanolic extract of *Dactyloctenium aegyptium* 200mg/kg inhibits the fungal growth and it is found that the Ethanolic extract of *Dactyloctenium aegyptium* at a dose of 200mg/kg and 100mg/kg leads to significant inhibition of fungal growth and it also inhibits the microbial growth in Aspargillus at a dose of 200mg/kg. Although the dose 100mg/kg showed less efficacy as the results shown in table 6 and figure 1.

Table 6: Antifungal Activity (Aspergillus niger)

S. No	Name of the compounds	Mean zone of inhibition (in mm)*	
		Aspergillusniger	
1	Griseoflavin	100µg	200µg
2	*Dactyloctenium aegyptium*	28	33

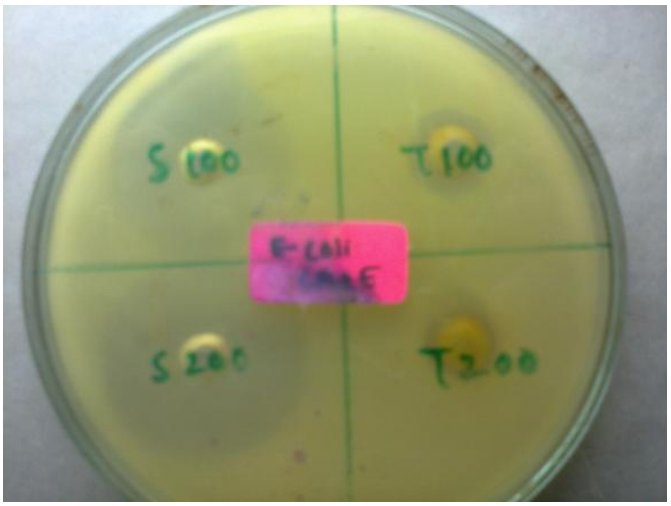

Figure 1: Zone of inhibition

4. Conclusion

In conclusion, the present study indicated a significant effect of the ethanolic extract of *Dactyloctenium aegyptium* and supports its traditional usage as an antimicrobial agent. Further, studies is required for the detailed studies in isolation of the compounds and pharmacological investigations of constituents, which have many pharmacological activity reported in traditionally and its exact mechanism of action and the results are confirm that *Dactyloctenium aegyptium* has a great potential as an Antimicrobial and may be useful in clinical conditions.

References

[1] Westh H, Zinn CS, Rosdahl VT et al. An international multicenter study of antimicrobial consumption and resistance in Staphylococcus aureus isolates from 15 hospitals in 14 countries. *Microb Drug Resist* 2004; 10: 169-176.

[2] Dean DA, Burchard KW. Fungal infection in surgical patients. *Am J Surg*., 1996; 171: 374-382.

[3] Gonzalez CE, Venzon D, Lee S et al. Risk factors for fungemia in children infected with human immunodeficiency virus: a case control study. *Clin Infect Dis* 1996; 23: 515-521.

[4] Cordell GA. Biodiversity and drug discovery-a symbiotic relationship. *Phytochemistry* 2000; 55: 463-480.

[5] Sieradzki K, Wu SW, Tomasz A. Inactivation of the methicillin resistance gene mice in vancomycin-resistant taphylococcusaureus. *Micro Drug Resist* 1999; 5: 253-257.

[6] Bandow JE, Brotz H, Leichert LIO et al. Proteomic approach to understanding antibiotic action. Antimicrob Agents Chemother 47: 2003, 948-955.

[7] Rojas R, Bustamante B, Bauer J et al. Antimicrobial activity of selected Peruvian medicinal plants. *J Ethnopharmacol* 2003; 88: 199-204.

[8] Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*) *Lebensm-Wiss u-Technol* 37: 2004; 263-268.

[9] Colombo ML, Bosisio E. Pharmacological activities of *Chelidonium majus* L (Papaveraceae). *Pharmacol Res* 1996; 33: 127-134.

[10] Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick J. ed. Perspectives on New Crops and New Uses. Alexandria, VA: ASHS Press; 1999: 457-462.

[11] Martins AP, Salgueiro L, Goncalves MJ et al. Essential oil composition and antimicrobial activity of three Zingiberaceae from S. Tome e Principle. *Planta Med* 2001; 67: 580-584.

[12] Krishnaraju AV, Rao TVN, Sundararaju D et al. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (Artemia salina) lethality assay. *Int J Appl Sci Eng* 2005; 2: 125-134.

[13] Balandrin MF, Kjocke AJ, Wurtele E et al. Natural plant chemicals: sources of industrial and mechanical materials. *Science* 228: 1985: 1154-1160.

[14] Tanaka H, Sato M, Fujiwara S. Antibacterial activity of isoflavonoids isolated from *Erythrina variegata* against methicillin resistant *Staphylococcus aureus*. *Lett Appl Microbiol* 2002; 35: 494- 498.

[15] Bakhru H.K.. "*Herbs That Heal"-natural remedies for health*. Nirali Prakashan, Pune, 17-18.

[16] Hussan Gilani and Atta-ur-Rahman."Trends in Ethnopharmacology". *J of Ethnopharmacology*; 2005; 100: 43-49.

[17] Memory Elvin- Levis. "Should we be concerned about herbal remedies". *J of Ethnopharmacology* 2005; 75; 141-164.

[18] Fantz, Paul R. "Ethnobotany of *Clitoria* (Leguminosae)". *Economic Botany* (New York Botanical Garden Press) 1991; 45 (4): 511–20.

[19] Pintusorn Hansakul, Chatri Ngamkitidechakul, Kornkanok Ingkaninan3, Seewaboon Sireeratawongand Watcharin Panunto. Apoptotic induction activity of *Dactyloctenium aegyptium* (L.) P.B. and *Eleusine indica* (L.) Gaerth. extracts on human lung and cervical cancer cell lines, *Songklanakarin J. Sci. Technol*. 2009; 31 (3): 273-279.

[20] Chandel R.S. & Rastogi R.P., *Phytochemistry*, 1980; 119: 1889-1902.

[21] Kumari Madhu, Phytochemical screening and antioxidant activity of *in vitro* grown plants *Dactyloctenium aegyptium* l., Using dpph assay, *Asian J Pharm Clin Res*, 2013; 6(2): 38-42.

[22] Sonali Nigam and P. N. Shrivastava, Phytochemical screening and antimicrobial activity of *Dactyloctenium aegyptium* leaves against Staphylococcus aureus, *Int J Pharm Bio Sci*, 2013; 4(3): (B): 963-968.

[23] Dwivedi Vivek, Chander B. Semwal , Narayan H Yadav, Evaluation of anti-ulcer activity of *Clitorea ternatea* Leaves (Linn) extract in Wistar rats, *Indian Journal of Research in Pharmacy and Biotechnology*, 2014; 2(3): 1225-1229.

[24] Jayachitra A., Krithiga N. and A. Rajalakshmi, Study on phytochemical and its antifungal activity in selected medicinal plants, *Inter J of Bio & Pharma Res*, 2012; 3(7): 890-898.

[25] Anand S. P., Doss A. and Nandagopalan V., Antibacterial studies on leaves of Dactyloctenium aegyptium Linn. - A high potential medicinal plant, *Inter J of Appl Bio and Pharma Tech*, 2011; 2(3): 453- 456.

[26] Rabeta, M. S. and An Nabil, Z., Total phenolic compounds and scavenging activity in *Dactyloctenium aegyptium* and *Vitex negundo* linn, *Inter Food Res J*, 2013; 20(1): 495-500.

[27] Subramanian M.S. and Prathyusha P., Pharmaco-Phytochemical Characterization of *Dactyloctenium aegyptium* Linn., *Inter J of PharmTech Res*, 2011; 3(1): 606-612.

[28] Indian Pharmacopia 1995.

[29] Selvamaleeswaran Ponnusamy, Wesely Ebenezer Gnanaraj, Johnson Marimuthu Antonisamy, Velusamy Selvakumar, Jeyakumar Nelson, The effect of leaf extracts of *Dactyloctenium aegyptium* Linn against the fish pathogens, *Asian Pacific J of Trop Med*, 2010; 412-420.