Special Fungal Pathogens Journal (SFPJ)

Special Fungal Pathogens Journal (SFPJ) 2016; Vol 1, No 1: p 0028-0031

SHORT COMMUNICATION

Antifungal activities of Cyclea paltata leave extracts in Tirunelveli, Tamilnadu, India

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Citation: Odaya Kumar P; Srinivasu K, Venkata Rao V, Onchweri AN, Muchiri JN. Antifungal activities of *Cyclea paltata* leave extracts in Tirunelveli, Tamilnadu, India. SFPJ; 1(1): 0028-0031

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Abstract

Background: *Cyclea peltata* extract has many important medical uses which makes it a potential extract for researchers prospecting for active compounds to provide intervention for the global antimicrobial pandemic especially in a resource-limited setting.

Objective: the objective is to determine if the extract also has antifungal properties:

Materials and Methods: Standard Microbiological Methods were adopted in processing the extract and analyzing for its antifungal potency.

Results: The leaf extract showed good activity against the tested Candida albicans and Aspergillus species raising the promise for its use as an efficient antifungal alternative.

Conclusion: *Cyclea peltata* showed antifungal activity and therefore opens a new horizon of studies into the spectrum of activities against fungal pathogens in addition to its known antibacterial activities

INTRODUCTION:

There are about 28 species of the genus *Cyclea peltata* made of climbing shrubs and which occur in the tropical regions of Asia. About 7 species are found in India. According to Kirtikar and Basu, (1), *Cyclea*

peltata (LAM) has some Pharmacognostic, antioxidant and antiulcer screening of *Cyclea peltata* roots. Vijayan, AH, Vohora, (2) has evaluated on the treatment and protective effect of *Cyclea peltata* on cisplatin-induced nephrotoxicity and oxidative damage. Hullatti and Sharada, (3) reported the diuretic activity on the root extract of *Cyclea peltata.* Lam-Latha *et al*., (4) evaluated the gastric anti-secretory and antiulcer activities of *Cyclea peltata* Lam. Kirana and Srinivasan, (5) has reported on the Type II diabetic activity on the roots aqueous extracts of *Cyclea peltata.*

On the other hand, Rukmani, (6) analyzed the nutritional and toxicological evaluation of *Cyclea barbeta* oil Kalyanasundaram, *et al.,* (7) reported on the biological active plant extracts of *Cyclea bicristata* as mosquito larvicides. Singh, (8) reported efficacy of plant extracts against *Cyclea ciliata.* It has been reported flavonoids and other medically important active compounds in *Cyclea ciliate* (9-10).

Much studies have also been conducted on the Gel and other extracts of *Cyclea barbata* Miers leaves including the chemical composition and gelling properties with the ultimate goal of outlining the best mixture that will be of most potency in the fight against tropical infections (11-12). The plant *Cyclea peltata* is speculated to possess various medicinal properties. A decoction of the leaves is employed in treatment of jaundice, asthma. Decoction of the roots used for the treatment of diabetes. Powdered roots are used in toothache. There is also speculation that the leaves of *Cyclea peltata* have medicinal properties related to Antiasthmatic property. *Cyclea peltata* also has an important place in indigenous medicine and in view of its usage, an attempt has been made to study the antimicrobial activities of this plant.

Aim:

To determine the antifungal activity of *Cyclea peltata* to validate its use as an alternative to imported antifungal drugs such as polyenes and azoles.

MATERIALS AND METHODS

Paper Discs impregnated with the known concentration of antifungal agents such as polyenes were placed on an agar plate that has been inoculated uniformly over the entire plate with a culture of the bacterium to be tested. The plate is incubated for 18 to 37 ^{0}C (for bacterium). For fungi the plate is incubated for 24 to 48 hours at 25^{0}C. During this period, the antifungal agent diffuses through the agar, and may prevent the growth of the organism. Effectiveness of susceptibility is proportional to the diameter of the inhibition zone around the disc. Organisms which grow up to the edge of the disc are resistant.

Materials required include: Whatman No: 2 filter paper of 6mm, Sabouraud Dextrose Agar plate (for Fungi), Forceps, Cotton swab, Standardized inoculums, Standard antibiotic disc, and Ethanolic extract of cylea paltata

Preparation of inoculum

The test fungal agents (Candida species and Aspergillus species) and the ethanolic extracts were obtained from National Chemical Laboratory (NCL) Pune, India, for this experiment, and were maintained by periodical susceptibility on Sabouraud Dextrose Agar (SDA). These fungal strains were inoculated in Sabouraud Dextrose broth and then inoculated at 37 ^{0}C & 25 ^{0}C for 6 to 8 hours.

Standardization of inoculums

Reproducibility of the disc-diffusion test largely depends on the size of the inoculums used. The zone of inhibition decreases with increasing size of the inoculums, because the antifungal agent has to react with a

greater number of fungi. Hence the inoculums size should be standardized, standardization of inoculum is done by comparing with the turbidity of the inoculum. The standard roughly compared with 1×10 organisms/ml, or 2 organisms seen on the smear under oil immersion objective.

Preparation of Standard:

Mixed 0.5 ml of 1.175%(w/v) hydrated barium chloride ($BaCl_2$ $2H_2O$), with 99.5 ml of 1% (w/v) or 0.36 N sulphuric acid (H_2SO_4).The resultant suspension of barium sulphate precipitate is used as the standard (1×10^8cells/ml). The standard was distributed in screw-capped tubes of the same size as those used in growing the broth culture which contains approximately 4 to 6 ml per tube. The standard was shaken before comparing with the test during an experiment and the standard was replaced every 6 months to ensure reliable and reproducible results.

Preparation of media

Sabouraud Dextrose Agar plates were used for the disc diffusion technique. The medium was prepared by adding the powder, dissolved by gently heating at 6.8, and by autoclaving at 121^0C for 15 to 50 minutes. The sterilized medium was cooled at 50^0C and poured into large culture plates to solidify at room temperature.

Procedure for disc diffusion techniques

The plates were labeled with the name of the culture, sample, and standard and with any specification required. Sterile cotton wool was wrapped around a sterile wooden applicator stick and dipped into the fungal suspension. Excess fluid was removed by rotating the swab with firm pressure against the inside of the tube above the fluid level. The inoculum was rubbed gently over the plate in several directions to obtain uniform distribution of the inoculums. Fine pointed pair of forceps was flamed on alcohol for sterility and cooled. The sterile disc was held with forceps and placed on the inoculated plate. (15mm from the edge of the plate and 24 mm in between the center of the discs). Five (5) discs were placed over the 10 cm diameter petri dish. The micropipette was used to load the antifungal sample in the sterile disc carefully. All plates were incubated at 37^0C in an incubator within 15 minutes after placing the discs. After the incubation, the diameter of the zones of inhibition of growth (including the 6mm diameter of the discs itself) was measured. Results reported as follows: Zone of clearance more than 12mm was taken as sensitive; zone 4 to 12 mm was taken as intermediate or sensitive dose-dependent while zone less than 4mm was interpreted as resistant.

Preparation of broth:

The commercially available powder for Sabouraud Dextrose Broth and Sabouraud Dextrose Agar was dissolved in distilled water by gentle heating and then they were allowed to boil and at the temperature of about 90 - 100^0C agar was added and stirred till the agar is completely dissolved. Then the pH was adjusted to 6.0. After the adjustment of pH, they were transferred to culture tubes (20 ml) and plugged with cotton, and sterilized at 121^0C for 15 minutes. After the sterilization process, they were removed from the autoclave and allowed to cool, when the temperature reaches 50^0C they were transferred to Petri dishes previously sterilized. Then the plates were stored in the refrigerator after leaving overnight at room temperature.

The plates were inoculated within 15 minutes. After preparing the inoculums, with a wax pencil the plate was divided into sections, according to the number of standard and sample solutions to be used, a sterilized

cotton swab was dipped into the nutrient broth. The excess fluid was removed by rotating the swabs with firm pressure against the inside of the tube above the third level. The well was made by the use of a borer. Test and control drugs were added to the cup plate by using a micropipette. The plates were incubated at 37 ^{0}C for 7 days in an incubator. The ethanolic extract of *Cyclea peltata* exhibited maximum antifungal activity against *Aspergillus nigra* (28 mm). From the results, it was observed that the Ethanolic extract of *Cyclea peltata* was found to exhibit significant Antifungal activity when compared with the standard drug Nystatin (100units/disc).

RESULTS AND DISCUSSION

Candida albicans and *Aspergillus species* were selected as representative of dimorphic opportunistic yeast. Although the test was controlled with the polyene group, further tests should be done with azoles to establish the spectrum of activity of this candidate antifungal agent. Further studies should also entail different strains of the fungi being tested.

Antimicrobial activity of *Cyclea peltata* leaf extracts

S/No	Name of the Microorganism	Zone of Inhibition in mm			
		S1	S2	S.C	Std
1.	*Candida albicans* (NCIM 3102)	23	22	8	25
3.	*Aspergillus niger* (NCIM 105)	28	15	5	30

S1-Ethanolic extract of *Cyclea peltata,* S2-Aqueous extract of *Cyclea peltata,* S.C-Solvent Control, Standard –Nystatin 100 units/ disc for fungi.

The plant *Cyclea peltata* is a medicinal plant used in many villages in developing countries. Although its use by the villagers is based on the assumption that it possesses various medicinal properties including antidiabetic and antibacterial but it is not clear if the extract also has an antifungal effect. As part of an ongoing global effort to discover new drugs from natural sources, the plant's leave extract was also selected for screening in prospecting for active compounds which may be of medicinal value in the treatment of common fungal infections. The fungus Candida and Aspergillus species were specifically selected because they represent the most common fungal agents of infection (13).

Cyclea peltata leaf extract is pungent and bitter, pungent in the post-digestive effect, and has hot potency. It possesses light and sharp attributes. It has bitter, digestant, antipyretic and astringent properties and is used in the diseases like fever, diarrhea, pruritus, dermatoses, worms, asthma, tumors, heart diseases, and wounds.

It is very interesting to note that the use of *Cyclea peltata* also includes treatment of wounds, and fever and priorities, because these are disease conditions known to be caused by the microbial agents including the fungi Candida and Aspergillus species. Our finding of the mean zone of clearance is different from Lakshmidevi and Jagadeep Chandra (14) who did not find any antifungal activity among the yeast isolates used. Human error, poor resources leading to poor asepsis and contamination, medicinal plant varieties, quality control, and standard operational procedures are some of the reasons why different laboratory's may produce spurious laboratory results which are

difficult to be clinically correlated to the condition of the patient (15-18).

This may be the case between our report of antifungal activity and no activity report of Lakshmidevi and Jagadeep Chandra (14) from India. This observation calls for more coordinated surveillance in a broader perspective. The antifungal activity of the *Cyclea peltata* methanoic extract may have some special policy implications

Cyclea peltata is a widely used drug in Ayurveda. Botanical source of the Laghupatha and Rajpatha are Cissampelos pareira and *Cyclea peltata* respectively, which belong to the Menispermaceae family. They contain many alkaloids like hyaline, hayatinine, hayatidine, and other bisbenzylisoquinoline alkaloids, be rberines, etc. which are found to be responsible for its various activities like anti-inflammatory, analgesic, antihaemorrhagic, gastroprotective, antioxidant, cardioprotective (18-20).

External application of the paste of its roots and leaves is extremely beneficial, in infected wounds, sinuses, and skin diseases like erysipelas and pruritus. The external application of this paste is said to be useful in serpent bite also. The root juice is salutary in headache, as nasal drops. The roots have anti-inflammatory activity and hence alleviates the edema. *Cyclea peltata* is a valuable wound healer and antidermatosis herb (19-20). Internal use of *Cyclea peltata* is a keen stimulant for the digestive system and endows the actions like an appetizer, digestant, astringent, vermicide, hence, is used in anorexia, dyspepsia, diarrhea, dysentery, worms, and abdominal pain (21-22).

In conclusion: The expression of antifungal activity by the ethanoic extract of *Cyclea peltata* in promising as antifungal alternative to imported and sometimes synthetic drugs. Futhere studies to confirm the spectrum of activity and safety considerations are strongly recommended.

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