

Asian Journal of Research in Biological and Pharmaceutical Sciences

Journal home page: www.ajrbps.com



ANTI-BACTERIAL ACTIVITIES OF AQUEOUS AND ETHANOLIC *CYCLEA PELTATA* LEAVE EXTRACTS IN TIRUNELVELI, TAMILNADU, INDIA

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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 in an effort to provide intervention for the global antimicrobial pandemic especially in resource limited setting. **Objective:** The objective is to determine and evaluate whether the aqueous and ethanolic extract has same antibacterial properties. **Material and Methods:** Pharmacognosical and Microbiological Methods were adopted in processing the extract and analyzing for its antibacterial potency. **Results:** The leaf extract showed good activity against the tested microorganism species raising for promise for its use as efficient antibacterial alternative and Ethanolic extract show better antibacterial effect compared to aqueous extract. **Conclusion:** Ethanolic extract of *Cyclea peltata* showed better antibacterial activity.

KEYWORDS

Cyclea peltata, Antibacterial activity and Aqueous and Ethanolic extract.

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INTRODUCTON

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 KR *et al.*,¹ *Cyclea peltata* (LAM) has some Pharmacognostic, antioxidant and antiulcer screening of *Cyclea peltata* roots. Vijayan *et al.*,² has evaluated on the treatment and protective effect of *Cyclea peltata* on cisplatin-induced

nephrotoxicity and oxidative damage. Hullatti and Sharada³ 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 *et al.*,⁵ has reported on the Type II diabetic activity on the roots aqueous extracts of *Cyclea peltata*.

On the other hand, Rukmani *et al.*,⁶ analysed the nutritional and toxicological evaluation of *Cyclea barbata* oil. Kalyana sundaram *et al.*,⁷ reported on the biological active plant extracts of *Cyclea bicristata* as mosquito larvicides. Singh *et al.*,⁸ reported efficacy of plant extracts against *Cyclea ciliata*. Omar⁹, reported flavonoids in *Cyclea ciliata*. Harraz *et al.*,¹⁰ isolated dammaran triterpenes from *Cyclea barbata*. Alyand Badran¹¹ reported the mosquito control with extracts from plants of the Egyptian eastern desert including *Cyclea gracillima*.

Apasara Arkarapanthu *et al.*,¹² investigated the Gel extracted from *Cyclea barbata* Miers leaves chemical composition and gelation properties. Iskandar Muda *et al.*,¹³ reported on the protective effect of *Cyclea barbata* Miers leaves against induced gastric ulcer in mice. Jian-Zhongwant *et al.*,¹⁴ isolated cytotoxic bisbenzylisoquinoline alkaloids from the roots of *Cyclea racemosa*.

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 treatment of diabetes. Powdered roots used in toothache¹⁶. There is also a 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 and evaluate the antibacterial activity between Ethanolic and aqueous extract of *Cyclea peltata*, so as to validate its use as alternative to other anti-bacterial drug.

MATERIAL AND METHODS

Paper Discs impregnated with known concentration of antibacterial agents ciprofloxacin 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°C (for bacterium). During this period, the antimicrobial 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 included Whatman No.2 filter paper of 6mm, Muller Hinton Agar plate, Forceps, Cotton swab, Standardized inoculums, Standard antibiotic disc and Ethanolic extract of *Cyclea peltata*.

Preparation of Ethanolic Extract

The dried coarse powder of *Cyclea peltata* was extracted successively with solvent of increasing polarity. Powder of *Cyclea peltata* was extracted with 2 litres of petroleum ether, chloroform and acetone respectively by continuous hot percolation method using soxhlet apparatus. After 24 hours when the extraction was completed, solvents were redistilled. Dried marc left after the acetone extraction is then again percolated in soxhlet apparatus with 2 litres of ethanol. After the completion of the extraction it was filtered and solvent was removed by distillation under reduced pressure, and the yellowish green colored extract was stored in a desiccator. The marc was dried for aqueous extraction.

Preparation of Aqueous Extraction:

The marc left after ethanol extraction was dried and macerated with 2-3 liters of chloroform water (0.25%) in a narrow mouthed bottle for three days. It was filtered and the solvent was removed by distillation under reduced pressure. The extract was then stored in desiccators.

Preparation of inoculum

The test bacterial agents (*Staphylococcus aureus*, *Bacillus subtilis*, *E.coli*, *Pseudomonas aeruginosa*, *Shigellashigae*, *Salmonella typhi*, *Salmonella Paratyphi* and *Proteus vulgaris*) are obtained from the National Chemical Laboratory (NCL) pune, for this experiment and maintained by periodical susceptibility

on nutrient Agar. These fungal strains were inoculated in nutrient agar broth and then inoculated at 37°C and 25°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 antimicrobial agent has to react with a greater number of bacteria. 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 1×10^8 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 ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$), 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 (1×10^8 cells/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. Shaken the standard before comparing and replaced the standard every 6 months.

Preparation of media

A Muller Hinton Agar plates were used for the disc diffusion technique. The medium was prepared by adding the powder, dissolved by gently heating at pH 7.2 and by autoclaving at 121°C for 15 to 20 minutes. The sterilize medium was cooled at 50°C and poured in large culture plates to solidify at room temperature.

Procedure for disc diffusion techniques

The plates were labelled with name of the culture (sample and standard) and with any specification required. Sterile cotton wool were wrapped round a sterile wooden applicator stick and dipped into the bacterial suspension. Excess fluids were removed by rotating the swab with a 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 were flamed on alcohol for sterility and cooled. The sterile disc was held with the 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 petridish. The micropipette was used to load the antibacterial sample in the sterile disc carefully. All plates were incubated all the plates at 37°C 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 follow: Zone of clearance more than 12mm was taken as sensitive; zone 4 to 12 mm were taken as intermediate or sensitive dose dependent while zone less than 4mm were interpreted as resistant.

Preparation of broth

The commercially available powder for Muller Hinton Broth dissolved in distilled water by gentle heating and then they were allowed to boil and at the temperature of about 90 -100°C agar was added and stirred till the agar get completely dissolved. Then the pH was adjusted to 7.4. After the adjustment of pH they were transferred to culture tubes (20 ml) and plugged with cotton and sterilized at 121°C for 10 minutes. After the sterilization process they were removed from the autoclave and allowed to cool, when the temperature reaches 50°C they were transferred to petridishes previously sterilized. Then the plates were stored in refrigerator after leaving overnight at the room temperature.

The plates were inoculated within 15 minutes. After preparing the inoculums, with a wax pencil the plate was divided into section, according to the number of standard and sample solutions to be used, 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 third level. The well was made by the use of borer. Test and control drugs were added into the cup plate by using micropipette. The plates were incubated at 37°C for 7 days in incubator. The ethanolic extract of *Cyclea peltata* exhibited maximum antibacterial activity against standard test microorganism. From the results, it was observed that the Ethanolic extract of *Cyclea peltata* was found to exhibit significant Antibacterial activity than aqueous extract as compared with the

standard drug ciprofloxacin (5mg/disc) for microorganism (Figure No.1-8). The presence of either alkaloids, phytosterols compounds of *Cyclea peltata* may be responsible for anti-bacterial activity.

RESULTS AND DISCUSSION

The plant *Cyclea peltata* is a medicinal plant used in many villages in developing countries. Although its use by the villagers is based the assumption that it possess various medicinal properties including anti-diabetic and antibacterial *Cyclea peltata* leaf extract is pungent and bitter in taste, 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, diarrhoea, pruritus, dermatoses, worms, asthma, tumors, heart diseases and wounds.

It is very interesting to note that the use of *Cyclea peltata* also include treatment of wounds, and fever and prurities, because these are disease conditions known to be caused by the microbial agents.

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 patient¹⁷⁻¹⁸. *Cyclea peltata* is a widely used drug in Ayurveda. Botanical source of the Laghupatha and Rajpatha are *Cissampelospareira* 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, anti-haemorrhagic, gastro protective, antioxidant, cardioprotective¹⁸⁻²⁰.

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 root has anti-inflammatory activity and hence alleviates the edema. *Cyclea peltata* is a valuable wound healer and anti-dermatosis herb¹⁷. Internal use of *Cyclea peltata* is a keen stimulant for digestive system and endows the actions like appetizer, digestant, astringent, vermicide, hence, is used in anorexia, dyspepsia, diarrhea, dysentery, worms and abdominal pain²⁰⁻²².

Table No.1: Antibacterial activity of *Cyclea peltata*

S.No	Name of the Microorganism	Zone of Inhibition in mm			
		S1	S2	S.C	Standard
1	<i>Staphylococcus aureus</i> (NCIM 2079)	20	13	9	35
2	<i>Bacillus subtilis</i> (NCIM 2063)	18	12	8	40
3	<i>E.coli</i> (NCIM 2065)	15	12	5	38
4	<i>Pseudomonas aeruginosa</i> (NCIM 2036)	20	15	-	40
5	<i>Shigellashigae</i> (NCIM 2024)	13	20	9	30
6	<i>Salmonella typhi</i> (NCIM 2023)	18	16	7	32
7	<i>Salmonella Para typhi</i> (NCIM 2022)	18	16	8	31
8	<i>Proteus vulgaris</i> (NCIM 2027)	15	14	6	26

Anti-bacterial activity of *Cyclea peltata* leaf extracts

S1-Ethanollic extract of *Cyclea peltata*, S2- Aqueous extract of *Cyclea peltata*, S.C- Solvent Control, Standard - Ciprofloxacin 5µg/disc for bacteria

ANTI MICROBIAL ACTIVITY OF EXTRACTS OF *CYCLEA PELTATA*



Figure No.1: Showing resistant pattern of Ethanolic and Aqueous extracts against *E.Coli*

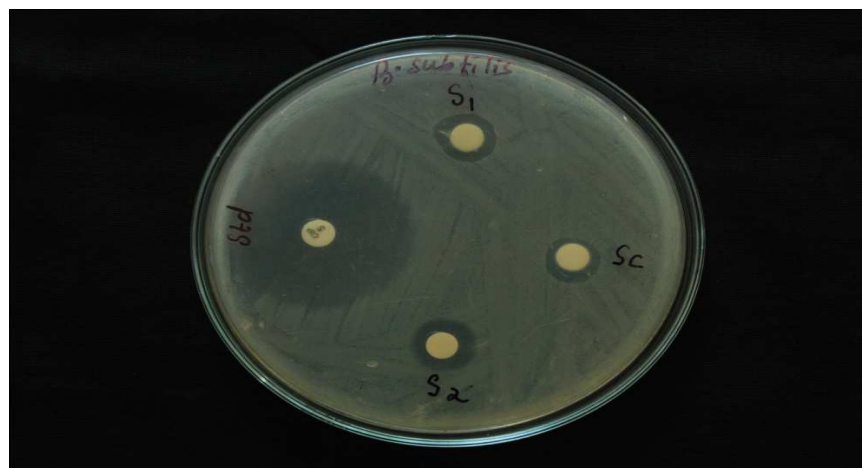


Figure No.2: Showing resistant pattern of Ethanolic and Aqueous extracts against *Bacillus subtilis*



Figure No.3: Showing resistant pattern of Ethanolic and Aqueous extracts against *Shigellashigae*



Figure No.4: Showing resistant pattern of Ethanolic and Aqueous extract against *Salmonella typhi*



Figure No.5: Showing resistant pattern of Ethanolic and Aqueous extract against *Staphylococcus aureus*



Figure No.6: Showing resistant pattern of Ethanolic and Aqueous extract against *Pseudomonas aeruginosa*



Figure No.7: Showing resistant pattern of Ethanolic and Aqueous extract against Proteus Vulgaris



Figure No.8: Showing resistant pattern of Ethanolic and Aqueous extract against Salmonella Para typhi

CONCLUSION

The expression of antibacterial activity by the ethanolic extract of *Cyclea peltata* was found to exhibit significant antibacterial activity than aqueous extract as compared with the standard drug ciprofloxacin (5mg/disc). Further studies to confirm the spectrum of activity and safety considerations are strongly recommended.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Jacob Raja Chairman SA Raja Pharmacy College, Tamilnadu, India for providing necessary facilities to execute this work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Kirtikar K R, Basu B D. Indian Medicinal plants, Bishan Singh Mahendra Pal Singh, Dehra Dun, 1993, 186-187.
2. Vijayan A H, Vohora S B S. Cisplatin-induced nephrotoxicity and oxidative damage of *Cyclea peltata* leaves, *Journal of Herbs, Spices and Medicinal Plants*, 6(3), 1996, 75-83.
3. Hullatti K K, Sharada M S. Diuretic activity on the root extracts of *Cyclea peltata* Lam, *Journal of the Indian Chemical Society*, 75(8), 2000, 485.
4. Latha G, Sudhakar P. Gastric antisecretory and antiulcer activities of *Cyclea peltata* Lam, *Indian Chem. Soc.*, 59(8), 2001, 1007-1008.

5. Kirana H, Srinivasan M. Type II Diabetic activity on the roots aqueous extracts of *Cyclea peltata*, *Indian drugs*, 38(6), 2010, 332-335.
6. Rukmani C. Nutritional and toxicological of *Cyclea barbata* oil, *Journal of Medicinal and Aromatic Plant Sciences*, 25(4), 1983, 1013-1019.
7. Kalyanasundaram M, Gomathy H, Govindhan S. Biological active plant extracts of *Cyclea bicristata* as mosquito larvicides, *Journal of Natural Remedies*, 3(1), 1986, 49-53.
8. Sinha R K. Ethno botany - The Renaissance of Traditional Herbal Medicine, *Ina Shree Publishers*, 242(1), 1996, 45-49.
9. Omar A A. Flavonoids in *Cyclea ciliata*, *Hamdard Medicus*, 43(1), 1994, 5-7.
10. Henry A N, Kumara, G R, Chitra V. Flora of Tamilnadu, India, *Botanical Survey of India, Southern Circle, Coimbatore, India*, 3, 1987, 258.
11. Apsara A, Yadava P S. Gel extracted from *Cyclea barbata* Miers Leaves, *Indian Journal of Traditional Knowledge*, 9(2), 2005, 515-520.
12. Kamraj V P. Herbal Medicine, *Current Sciences*, 78(1), 2000, 35-39.
13. Agwu E, Ihongbe J C, McManus B A, Moran G P, Coleman D C, Sullivan D J. Distribution of yeast species associated with oro-pharyngeal candidiasis in HIV-infected patients in Southwest Uganda, *Medical Mycology*, 50(3), 2012, 276-280.
14. Lakshmi Devi N, Jagadeep Chandra S. Evaluation of in vitro Antimicrobial Activity of *Caesalpinia Bonducella* and *Cyclea Peltata* Extracts against opportunistic Microbes Isolated from Wounds in Diabetic Patients, *Asian Journal of Phytomedicine and Clinical Research*, 3(2), 2015, 55-63.
15. Angerhofer C K, Guinaudeau H, Wongpanich V, Pezzuto J M, Cordell G A. Antiplasmodial and cytotoxic activity of natural bisbenzylisoquinoline alkaloids, *Journal of Natural Products*, 62(1), 1999, 59-66.
16. Encyclopedia of Ayurvedic Medicinal Plants (A Candle of Medicinal Herb's Identification and Usage), *PATHA – Cyclea peltata, Cissampelos parietal*, 2012.
17. Meena J, Santhy K S. Efficacy of methanol extract of *cyclea peltata* as a potent anticancer equivalent, *European Journal of Environmental Ecology*, 2(2), 2015, 65-71.
18. Singh S, Nishteswar K. Review on *Cissampelos Pareira* and *Cyclea Peltata* (Patha Dwaya) Phyto-Pharmacological Perspectives, *International Journal of Ayurvedic Medicine*, 4(4), 2013, 282-289.
19. Singh J. Efficacy of plant extracts against *Cyclea ciliata*, *Bulletin of Medico-Ethnobotanical Research*, 19(3-4), 1994, 165-175.
20. Pathek MA, Farrington D, Jr, Fitzpatrick TB. The presently known distribution of Furocoumarins (Psoralens) in Plant, *Department of Dermatology of the Harvard Medical School, New York*, 1961, 34-39.
21. Jyothi Abraham. Dennis Thomas Antimicrobial properties of *Cyclea peltata* leaves, *Asian Pacific Journal of Tropical Disease*, 70(5), 1999, 517-520.
22. Sofowora A. Medicinal Plants and Traditional Medicine in Africa, *1st Benin City: John Wiley and Sons Ltd*, 131, 1982, 168-171.

Please cite this article in press as: P. Odaya kumar. et al. Anti-bacterial activities of aqueous and ethanolic *cyclea peltata* leave extracts in Tirunelveli, Tamilnadu, India, *Asian Journal of Research in Biological and Pharmaceutical Sciences*, 4(2), 2016, 60-67.