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Phytochemical Screening And In-Vitro Anti-Microbial Activity of Various Extracts of Cyclea Peltata Lam.

Mathew George, Lincy Joseph, Christy K. Jose*

Abstract:-Most of new biologically active compounds are natural products. British National Formulary (BNF) has been estimated to contain 56% of the lead compounds for medicines are natural products or are derived from natural products. In all the traditional systems of medicines, herbal drugs constitute a major part. *Cyclea peltata* (Patha) is one of the herbs mentioned in all ancient scriptures of Ayurveda. In Ayurveda the plant is used for the treatment of fever, urinary problems and skin infections. The roots have great medicinal value and are used for medicinal purpose, both, internally as well as externally. It is traditionally used in many cases such as treatment of fever, urinary problems and skin infections. It is observed from the review of literature that only few biological and chemical studies have been done on *Cyclea peltata*. Therefore, it seems to be essential to have a detailed screening on the pharmacological actions of this plant to exploit the various potentialities. The present study was carried out to find the various phtyochemicals in the extracts prepared and to screen its *in-vitro* antimicrobial activity. The fully dried roots were powdered using mixer grinder, sieved through 40 mesh and subjected to soxhlet extraction using solvents - petroleum ether, chloroform, ethyl acetate, methanol and distilled water. The percentage yield was calculated and were subjected to preliminary phytochemical analysis for – saponins, tannins, triterpenes, alkaloids and flavonoids.

In-vitro antimicrobial activity studies were conducted using samples of the prepared extracts. The agar diffusion method was followed for this. Bacterial species like Shigella dysentriae, Bacillus subtilis, Escherichia coli, Klebsiella Pneumoniae, Staphylococcus aureus, Salmonella choleraesuis and fungal species like Candida albicans and Tinea capitis were the organisms tested. The plant showed significant antibacterial and antifungal activity against almost all the organisms and especially good activity was found against Staphylococcus aureus and dermatophytes.

Keywords:-Cyclea Peltata, Preliminary Phytochemical Analysis, In-Vitro Antimicrobial Activity.

I. INTRODUCTION

From time immemorial, plants are being used in medicine because they have fitted the immediate personal use, they are accessible and inexpensive, the practitioners speak to those who have used them in their own language and they are not provided from a professional or government apparatus. The use of plants for medicines around the world vastly exceeds that of modern synthetic drugs.

New biologically active compound are mostly obtained from natural products. 56% of the lead compounds for medicines in the BNF are natural products or are compounds that are derived from natural products (Midley, 1988). Semi-synthetic preparation of many drugs uses plant products as starting material such as the use of plant steroids for the manufacture of oral contraceptives and other steroidal hormones.

Cyclea peltata (Patha) is one of the herbs mentioned in all ancient scriptures of Ayurveda. The great sage Charaka has categorized patha as sandhaniya – a healing herb; stanyasodhana – lactodepurant, jvarahara – alleviates fever. Maharishi Susruta has mentioned it as visaghna – anti – toxin (Susruta Samhita, Sutra, A-38) and also to be useful (especially, the leaves) in diabetic disorders of the skin, like boils.

Cyclea peltata belongs to the family Menispermaceae. The plant is a slender twining shrub and grows throughout India and Sri Lanka. It has simple, alternate, heart shaped leaves, which are 2.5-10 cm long and 2.5-3.75 cm broad, stipules 5-10 cm long and nerves 7-11 (Fig. 1:a-c). The flowers are pale yellow, unisexual, in axillary panicles and the plant blooms in the rainy season. It has fruits that are ovoid drupes, brown or scarlet in colour and the seeds are covered. It has greyish brown tuberous roots which are cylindrical, irregularly curved.

In Ayurveda, Cyclea peltata is used for ailments like fever, urinary problems and skin infections (Yoganarasimhan, 2002). Its roots have great medicinal value and are used both, internally and externally. Its roots and leaves are extremely

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beneficial, in infected wounds, sinuses, and skin diseases. The external application of root and leaf paste is reported to be useful in serpant bite also. The roots have anti-inflammatory activity. The plant is a valuable wound healer and anti-dermatitis herb.

Eleven quaternary alkaloids are isolated from root bark, three of which were termed menismine, cissamine and pareirine. five more tertiary alkaloids present in root bark. From the leaves cycleanine, bebeerines, hayatinin, hayatidin, hayatin and querticol were isolated.

Fig. 1.1. a-c: Habit of *Cyclea peltata* a: Twining branch, b: Twining branch with fruits, c: uprooted plant showing rhizomatous roots

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II. REVIEW OF LITERATURE

A. *Preliminary Phytochemical Investigation*

In Ayurveda species of the family Menispermaceae are used for the treatment of fever, urinary problems and skin infections (Yoganarasimhan, 2002). *Cyclea peltata* Lam is one of them and are found in Western Ghats and Deccan region (Saldanha, 1984; Warrier, 1994). Reports have shown that the plant has various alkaloids and have different pharmacological activities. Five bisbenzylisoquinoline alkaloids - cycleapeltine, cycleadrine**,** cycleacurine**,** cycleanorine**,** and cycleahornine chloride are isolated from this plant(Kupchan *et al.*, 1973). Bisbenzylisoquinoline alkaloids have been reported to have antiplasmodial and cytotoxic activities (Angerhofer *et al*., 1999) and antilithiatic activity (Christina *et al*., 2002). *Cyclea peltata* have modulatory effect on stone formation induced by ethylene glycol treatment in rats (Christina *et al.,* 2002). Studies have reported on the protective effect of *Cyclea peltata* on cisplatin-induced nephrotoxicity and oxidative damage. Post-treatment of *Cyclea peltata* extract might effectively ameliorate the oxidative stress parameters observed in cisplatin induced renal toxicity and could be used as a natural antioxidant against cisplatin-induced oxidative stress (Fijesh *et al.,* 2007).

Various government and private agencies have initiated many screening programs of the natural resources. The first screening program among these was by the Central Drugs Research Institute (CDRI) initiated in 1964 which spanned a period of 25 years and around 2500 plants with a variety of biological activities were screened. The European Trade Association formed the European Scientific Co-Operative of Phytotherapy (ESCOP) under the auspicious of the European Economic Community (EEC) to advance the state of herbal medicine. A series of plant species monograph is published by ESCOP for EEC marketing authorization (David, 1996). Various organizations like WHO and UNICEF are interested in plants to be used for the treatment of various diseases of children.

B. *In-Vitro Antimicrobial Activity*

Few reports have shown that polar extracts have inhibited the growth of both Gram-positive and Gram-negative bacteria (Masika and Afolayan, 2002; Karaman et al., 2003; Sharma et al., 2010; Dalal et al., 2010). Nayak et al. (2008) observed that the crude extract of the plant has antimicrobial activity against Staphylococcus aureus, Klebsiella Pneumoniae and Escherichia coli.

III. MATERIALS & METHODS

A. *Collection of Plant Materials*

Cyclea peltata plants were collected from different places of Pathanamthitta District, Kerala. The collected plants were authenticated by Dr. V.T. Antony, Associate Professor, St. Berchman's College Changanacherry, Kottayam, Kerala. Roots were removed from the plants, washed thoroughly in water and dried at room temperature. Dried roots of more than 1 kg were collected.

- *Preparation of Extract*

The fully dried roots were powdered using mixer grinder, sieved through 40 mesh and subjected to soxhlet extraction using different solvents. The solvents for extraction were petroleum ether, chloroform, ethyl acetate, methanol and distilled water. The extracts were filtered and concentrated to dryness *in vacuo* at 40^{o}C.

- *Percentage yield*

The percentage yield was calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{(Weight of the product obtained after evaporation)}}{\text{Weight of the powdered sample taken initially}} \times 100$$

B. *Preliminary Phytochemical Investigation*

- *Test for Saponins*

The formation of frothing indicates the presence of saponins when extract (300 mg) was boiled with 5 ml water for two minutes; the mixture was cooled and mixed vigorously and left for three minutes.

- *Test for Tannins*

An aliquot of the extract (dissolved in water) was added with 2 ml of sodium chloride (2%), filtered and mixed with 5 ml 1% gelatin solution. Precipitation indicates the presence of tannins

- *Test for Triterpenes*

Extract (300 mg) was mixed with 5 ml chloroform and warmed for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well. The appearance of red colour indicates the presence of triterpenes (Harborne, 1973).

- *Test For Alkaloids*

Extract (300 mg) was digested with 2 M HCl, and the acidic filtrate was mixed with amyl alcohol at room temperature. Pink colour of the alcoholic layer indicates the presence of alkaloids (Harborne, 1973).

- *Test For Flavonoids*

The presence of flavonoids was determined by using 1% aluminium chloride solution in methanol, concentrated HCl, magnesium and potassium hydroxide solution

C. Antimicrobial Activity

Bacterial species like Shigella dysentriae, Bacillus subtilis Escherichia coli, Klebsiella Pneumoniae, Staphylococcus aureus, Salmonella choleraesuis and fungal species like Candida albicans and Tinea capitis were the organisms tested. These were collected from stock cultures of Rubber Research Institute of India, Kottayam, Kerala. The bacterial strains were obtained as fresh colonies grown on Mac Conkey and blood agar plates. The sensitivity testing was done using Muller Hinton Agar plates. Known volume of bacterial suspension was transferred to each microplate well. Ten micro liters of the Cyclea peltata root extract dissolved in sterile water (200 µg/ml) was added to the microplate wells and incubated at 35-37°C for 48 hrs. Results were analyzed visually on the basis of turbid zone of inhibition. [+ = bacterium colonies deposited in the bottom of the well, ++ = turbidity with bacterium.

The antimicrobial activity was also evaluated by the agar diffusion method (Murray *et al*. 1983). Bacteria were cultured overnight at 37 °C in Mueller Hinton Broth (MHB, Oxoid) and fungi at 28 °C for 72 h in Potato Dextrose Broth (PDB, Oxoid) and used as inoculum. A final inoculum, using 100: l of suspension containing 108 CFV/ml of bacteria and 104 spore/ml of fungi spread on Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) medium, respectively. The disc (6mm in diameter) was impregnated with 10 µl of 100 mg/ml (1 mg/disc) extracts placed on seeded agar. Gentamicin (10µg/disc), streptomycin (10 µg/disc) and tetracycline (10µg/disc) were used as positive controls for bacteria and fluconazole (10 µg/disc) and ketoconzole (10 µg/disc) for fungi. The test plates were incubated at 37 °C for 24 hrs for bacteria and at 28 °C for 72 hrs for fungi depending on the incubation time required for a visible growth. MIC values were also studied for microorganisms, which were determined as sensitive to the extract in disc diffusion assay. Sterile filter paper discs (6mm in diameter) containing 2.5–1000 µg/disc of plant extracts were placed on the surface of a medium. MIC was defined as the lowest concentration of extract that inhibited visible growth on agar.

IV. RESULTS

A. Phytochemical Studies

- *Percentage yield*

The percentage yield, nature and colour of various extracts of Cyclea peltata was calculated and given in Table 1. From the percentage yield it was observed that the methanol extract gave the maximum amount of yield and the hexane extract gave the minimum amount of yield.

Sl #	**Extracts**	**Nature of extracts**	**Colour Yield**	**(% w/w)**
1	*Petroleoum Ether*	Semisolid	Dark green	17.5
2	Chloroform	Semisolid	Dark green	9.2
3	Ethyl acetate	Semisolid	Dark green	14.6
4	Methanol	Semisolid	Dark green	19.6
5	*Aqueous*	Semisolid	Dark green	18.5

Table 1**:** Percentage yield of Various Extracts of Cyclea Peltata

B. Preliminary Phytochemical Analysis

The phytochemical analysis of the root extracts by qualitative study showed the presence triterpenoids and flavonoids and the absence of phytosterol, gums, mucilage, lignins and saponins. (Table2) From the phytochemical analysis it was observed that *Cyclea peltata* preliminarily contains amino acids, carbohydrates, alkaloids, lipids and glycosides.

Phyto- constituent	**Extracts**				
	Petroleoum Ether	Chloroform	Ethyl acetate	Methanol	*Aqueous*
Alkaloids	+	+	+	+	+
Reducing Sugar	-	-	-	+	+
Phytosterol	-	-	-	-	-
Fixed oil & Fats	-	+	+	-	-
Phenolic compounds & Tannins	+	+	+	+	+
Proteins & Amino Acids	-	+	-	+	-
Gums & Mucilage	-	-	-	-	-
Flavonoids	-	-	+	+	-
Lignin	-	-	-	-	-
Saponins	-	-	-	-	-

Table 2 Preliminary Phytochemical Screening of Various Extracts of Cyclea peltata

C. In- Vitro Antimicrobial Activities

The Results of in-vitro antimicrobial activity by sensitivity testing method is given in Table 3.

The disc diffusion method was used to determine zones of inhibition of Cyclea peltata extracts (organic and aqueous). The plant showed significant antibacterial and antifungal activity against almost all the organisms (Table 4) and especially good activity was found against Staphylococcus aureus and dermatophytes. However, the petroleum ether extracts of this plant showed little antimicrobial activity. Significant antimicrobial activity was observed in methanolic and aqueous extracts. Amongst the test organisms used, Staphylococcus aureus was found to be most sensitive; Tinea capitis came next, followed by Proteus mirabilis, Escherichia coli, Shigella dysentriae, Salmonella typhi, Candida albicans, and Bacillus subtilis. Increased inhibition was found at higher levels of extract concentration. . Some of the extracts like the methanolic extract of Cyclea peltata gave very low MIC values, and inhibited the growth of Escherichia coli, Proteus mirabilis and Staphylococcus aureus with a concentration of 2.5µg/disc. Since MIC of Cyclea peltata against Staphylococcus aureus was substantially low (2.5µg/disc).

Microorganisms		Turbidity patterns				
		Extract				
		Petroleoum Ether	Chloroform	Ethyl acetate	Methanol	*Aqueous*
Bacteria						
1	*Shigella dysentriae*	+	+	+	+	+++
2	*Bacillus subtilis*	-	-	-	-	-
3	*Escherichia coli*	++	++	+++	++	+++
4	*Klebsiella Pneumoniae*	-	-	-	-	-
5	*Staphylococcus aureus*	+	++	+	+	+
6	*Salmonella choleraesuis*	-	-	-	-	-
Fungal strains						
1	*Candida albicans*	-	-	-	-	-
2	*Tinea capitis*	++	+	+	+	++

Table 3: Antimicrobial activity of various extracts of *Cyclea peltata*

Microorganisms		*Petroleoum Ether*	Chloroform	Ethyl acetate	Methanol	*Aqueous*
Bacteria strains		MIC (µg/disc)	MIC (µg/disc)	MIC (µg/disc)	MIC (µg/disc)	MIC (µg/disc)
1	*Bacillus subtilis*	100	200	100	100	100
2	*Escherichia coli*	100	100	2.5	100	2.5
3	*Klebsiella pneumoniae*	200	100	10	100	10
4	*Proteus mirabilis*	100	100	2.5	5	2.5
5	*Staphylococcus aureus*	100	100	2.5	10	2.5
6	*Pseudomonas aeruginosa*	100	200	100	200	100
Fungal strains						
1	*Candida albicans*	100	200	10	100	10
2	*Tinea capitis*	100	100	2.5	100	5

Table 4: The MIC values (µg/disc) of *Cyclea peltata* Extracts against the Microorganisms

V. DISCUSSION

A. *Preliminary phytochemical investigation*

The preliminary phytochemical investigation of the root extracts showed the presence of amino acids, carbohydrates, alkaloids, lipids and glycosides.

B. *In-Vitro Antimicrobial Activity*

There are some reports showing that polar extracts inhibited the growth of both Gram-positive and Gram-negative bacteria (Masika and Afolayan, 2002). However, in the present study there was no difference between the polar and non polar extracts in the antimicrobial activity. In the present study also the aqueous extract showed this property.

The plant showed significant antibacterial and antifungal activity against almost all the organisms and especially good activity was found against *Staphylococcus aureus* and dermatophytes. The petroleum ether extracts of this plant showed little antimicrobial activity. Significant antimicrobial activity was observed in methanolic and aqueous extracts.

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