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Metabolite Extraction And Functional Annotation Of Cyclea Peltata From Western Ghats: Insights Into Ethnopharmacological Potential

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Abstract
Cyclea peltata is very important medicinal plant which is traditionally used in Vythiri thaluk, Wayanad district, Kerala, India against different diseases in human and animals. The objective of this study was to evaluate antimicrobial activity and characterize the chemical constituents in the extracts of the leaves of Cyclea peltata by using modern techniques like High Performance Liquid Chromatography (HPLC) and Liquid Chromatography–Mass Spectrometry (LC/MS-MS). The evaluation of antimicrobial activity of Cyclea peltata was determined by disc diffusion method. The extracts showed different degree of inhibitory potential against all the tested bacteria such as Escherichia coli and Pseudomonas aeruginosa and two gram-positive strains of Bacillus subtilis and Staphylococcus aureus. LC/MS-MS analyses showed that majority of identified compounds in Cyclea peltata are alkaloids, and flavonoids. The high percentage of compounds that were identified in the extracts of which are chemically and biologically important. In HPLC fingerprint Cyclea peltata showed total 5 major peaks to attribute as novel metabolite and active ingredients. Therefore, the identified good number of chemical compounds from methanol extract of the leaves of Cyclea peltata have many ecological benefits for different ailments. Result from this study suggested that the extracts of the leaves of Cyclea peltata could be used as a natural antimicrobial agent.
Keywords: *Cyclea peltate, Antimicrobial, Ecological, Medicine, HPLC, LCMS, Plants*

1. INTRODUCTION

People have utilised plants from the dawn of civilisation. Plants offer food, medicines, building materials, crafts, and tools, and a variety of other items such as fuel, paints, and poisons, among other things [1-2]. Currently, research is being done for human benefit on the chemical and pharmacological components of medicinal plants used in various traditional systems around the world [3]. With about 45,000 plant species and 550 tribal communities belonging to 227 ethnic groups inhabiting in distinct geo-climatic zones with various plant species and a rich traditional knowledge system, India has a diverse species of plants and a rich traditional knowledge system [4]. Tribal communities who live close to nature are stewards of a distinct system of conventional knowledge, A rich ethnomedical tradition, as well as knowledge of the environment's plants and animals. Because the bulk of these ethnic groups lack their own scripts and written languages, traditional tribal medicine information such as prescriptions, pharmacology, disease attitudes, and diagnosis is lost [5]. The World Health Organization (WHO) indicates that 80% of the population in developing nations depend on traditional medicine, primarily plant medicines [6]. No less than 25% of medications in use today are plant-based, with most synthetic medicines being modelled after plant prototypes. Therapies based on plants provide a disadvantage of increased safety and reduced costs as compared to synthetic medicines [7]. Plants are freely available and their by-products are biodegradable. Nevertheless, microbial genetic adaptability has been responsible for the worldwide dissemination of antimicrobial resistance [8]. Numerous contemporary medicines continue to utilize plant-derived active ingredients, yet phytochemical examination has only been conducted on a minority of the estimated 250,000–500,000 plant species, and only a small number have been subject to biological or pharmacological assessment [9-10]. Research indicates that plants employed by different ethnic groups have the ability to suppress the growth of disease-causing microbes [11-12], and numerous medicinal plants are being investigated for their antibacterial activity because of growing antibiotic resistance [13]. Hence, identified one novel plant *Cyclea peltata* from Vythiri Taluk of Wayanad district to investigate their chemical and biological properties to add in the various application of mankind.

MATERIALS AND METHODS

Study area

Vythiri Taluk of Wayanad district was chosen for the current study because of its high Phyto diversity. Field observations and sampling were conducted between December 2021 and April 2022. Wayanad, in Kerala's northeastern and administrative headquarters at Kalpetta, is found at an altitude of 700–2100 meters in the Western Ghats with an area of 2,131 km^2 (5.48% of the area of Kerala) between 11°27'–11°58'35\" N latitude and 75°47'50\"–76°26'35\" E longitude. It is famous for its rich medicinally valuable plants and dense tribal population, such as Kurichia, Kuruma, Kattunaika, Adiyan, and Paniya tribes. Vythiri, which is cooler than the rest of Wayanad, is at an elevation of approximately 700 meters above sea level. Its geology comprises Basic Rocks (36.69), Charnockite group (4214.93), Migmatite Complex (564.41), and Peninsular Gneissic Complex. It is geomorphologically characterized by Denudational Structural Hills (2504.99), Piedmont Zones (2049.37), Residual Hills (166.61), Rock Exposures (7.86), Valleys (132.09), and Water Bodies (8.86) [14]. Such biodiversity hotspots as Banasurasagar, Chembra Peak, Pookot Lake, and Soochipara Falls enrich the region's flora and fauna. Agriculture is the prime occupation, with crops such as paddy, areca nut, banana, ginger, pepper, cardamom, coconut, turmeric, tea, and coffee. Local wisdom reveals many species of plants remain unexploited, and modernization is posing a threat to ethnobotanical traditional wisdom [15]. Documentation and preservation of this precious knowledge possessed by ethnic communities are the need of the hour.

Figure 1: Study area: Vythiri, Wayanad, India

According to a preliminary survey on the usage of endemic medicinal plants, herbal medicine is still used to treat the majority of ailments in a few communities around this district. As a result, this research aimed to conduct a systematic investigation of the numerous less-exploited endemic species of this region. In consideration of the facts, current study medicinal plants from the selected area were *Cyclea peltata* of the plant family Menispermaceae

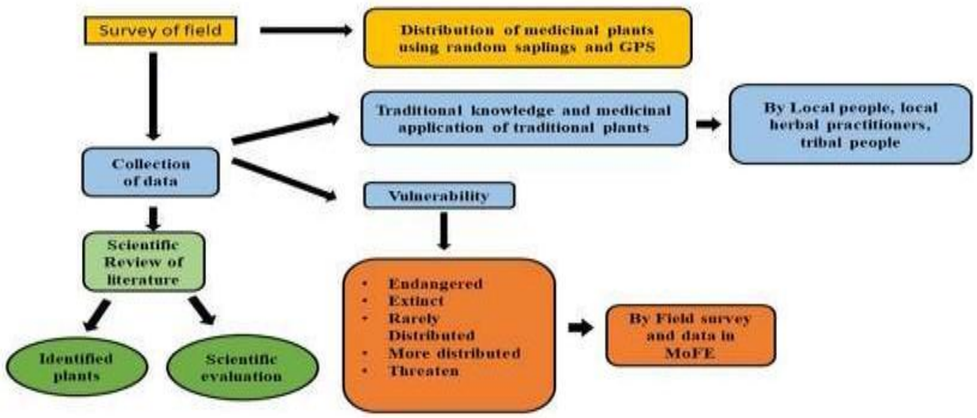

Figure 2: Schematic representation of the scientific survey of traditional knowledge and medicinal application of plant species.

The *Cyclea peltata* plant was whole-harvested from Wayanad's Western Ghats, primarily Vythiri Taluk, during February and preserved in sterile bags. Identification of the specimen was done with the assistance of review papers, professors, and botanists. Identification was followed by washing of samples thoroughly using distilled water for the removal of dust and shade-drying at room temperature. Dried leaf, stem, and root were then separated, keeping the primary focus on the leaf. The leaves were dried and then ground to a fine powder in a grinder for more than 20 seconds.

Figure 3: Plant sample preparation of *Cyclea peltata*

Plant extraction procedure for *Cyclea peltata*

Prepare a thimble of *Cyclea peltata* leaf powder (19875.0 mg) and place the thimble into the Soxhlet extractor. 500 ml of methanol was added into the Soxhlet extractor with the thimble and balanced into the round-bottom flask. By using a heating mantle, the round bottom flask with methanol was heated and evaporated. The evaporation process should be continued for 24 hours by using a Soxhlet extractor. So, after the extraction of *Cyclea peltata*, the extracted product was filtered, and the methanol solvent was evaporated completely from the system by using rotary evaporator and the balance solvent is taken in a standard flask and then add 10 ml of methanol to the solvent and pour it into the petri dishes and keep it for air dry.

Antibacterial Activity of *Cyclea peltata*

Antibacterial activity of *Cyclea peltata* leaf was tested by agar well diffusion assay with some modifications. Petridishes were prepared by pouring 20 mL of sterilized NA media under aseptic condition and allowed to solidify. After solidification of the media, 100 μL of standardized test microbial inoculums of Escherichia coli, *Pseudomonas aeruginosa, Bacillus subtillis* and *Staphylococcus aures* were spread uniformly using sterile cotton swabs. 6 mm diameter agar is drawn from plate to form a well using sterile cork borer. Antibiotic penicillin was used as positive control and DMSO as negative control. After keeping at 4°C for 4 hours for the diffusion of antibacterial metabolites, thereafter plates were incubated at 37 °C for 24h. The diameter of the inhibition zone around the well is measured in millimetre (mm) and the average of three repeated agar discs were taken to assess the strength of antibacterial activity.

RESULTS AND DISCUSSION

Antimicrobial analysis: disc diffusion method

Plants extract of *Cyclea peltata* were screened for antimicrobial potential by disc diffusion method. Two gram-negative organisms (*Escherichia coli* and *Pseudomonas aeruginosa*) and two gram-positive organisms (*Bacillus subtilis* and *Staphylococcus aureus*) were tested. The inhibition zones are presented in Table 1 and Figure 4. Both of the extracts presented greater inhibition zones at lower concentrations (10 and 20 μg/mL) than the reference penicillin drug. *Cyclea peltata* presented inhibition zones of 3.1 ± 0.03 mm against *E. coli*, 2.9 ± 0.04 mm against *P. aeruginosa*, 3.1 ± 0.02 mm against *B. subtilis*, and 3.2 ± 0.02 mm against *S. aureus* at 20 μg/mL. Results are more positive at 20 μg/mL than at 10 μg/mL (Table 1 and Figure 4).

Table 1. Antimicrobial activity of ***Cyclea peltata*** **against *E. coli, P. aeruginosa, B. subtilis,*** **and *S. aureus***

Zone of inhibition (in cm)				
Target	10 μg/mL	20 μg/mL	Standard	Negative control
E. coli	2.8 ± 0.01	3.1 ± 0.03	2 ± 0.01	NI
P. aeruginosa	2.5 ± 0.02	2.9 ± 0.04	2.5 ± 0.03	NI
B. subtilis	2.6 ± 0.01	3.1 ± 0.02	2.4 ± 0.02	NI
S. aureus	2.6 ± 0.03	3.2 ± 0.02	2.8 ± 0.01	NI
±: denotes the standard error; NI: no inhibition; Standard: 10 μg Penicillin; Negative control: saline solution. Values are means of triplicates				

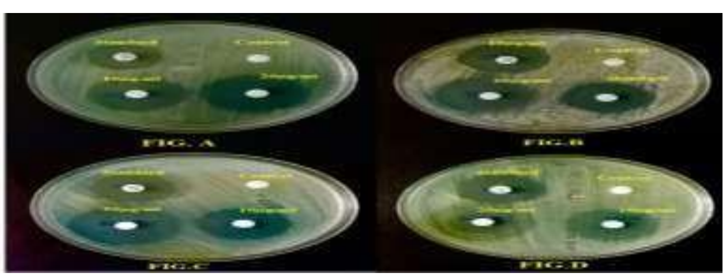

Figure 4: Antimicrobial activity of ***Cyclea peltata*** extract: Fig A. ***E. coli,*** Fig B. ***P. aeruginosa,*** Fig C. ***B. subtilis,*** Fig D. ***S. aureus***

HPLC analysis of *Cyclea peltata*

The HPLC fingerprinting and qualitative analysis of *Cyclea peltata* were done and is showcased in Fig.5. A total of 5 major peaks were extracted from the HPLC analysis with prominent retention factor. Peak 1 and 5 were two major peaks with R_t value 2.01 and 5.1 which corresponds to beta-myrcene and pinene respectively. Peak 2 have R_t value 3.14 which corresponds to isovaleric acid. Peak 3 have R_t value 3.28 which corresponds to hexenal and the peak 4 have R_t value 4.14 which corresponds to n-heptanal. And chemical composition is showcased in Table 2.

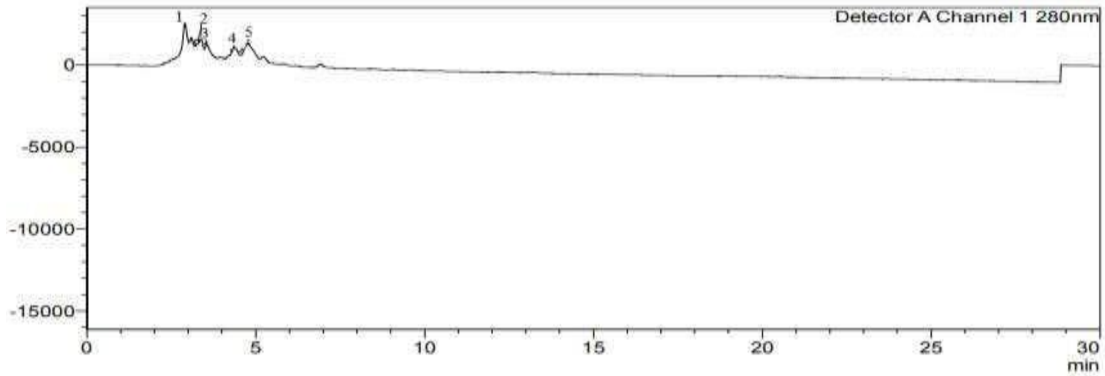

Figure 5: HPLC graph of *Cyclea peltata*

Table 2. Chemical composition of *Cyclea peltata*

Cyclea peltata			
Peak no.	Compound	Area	Retention time
1	beta-myrcene pinene	41517	2.01
2	isovaleric acid	14177	3.14
3	hexenal	27167	3.28
4	n-heptanal	23667	4.14
5	pinene	11438	5.1

LC/MS-MS analysis

LC/MS-MS analysis of *Cyclea peltata*

The representative of LC/MS-MS chromatograms of *Cyclea peltata* is illustrated in Fig. 6. The resultant peaks identified were 136.9, 121.9, 60.3, 166.1, 220.3, 316.1, 380.9 m/z values which corresponds to protocatechuic aldehyde (flavonoids), salicylic acid-o-hexoside (phenolic glycoside), toxifoli (flavonoid), protocatechuic aldehyde (flavonoids), canthin-6-one (alkaloid), laurycolactone b (Biphenylneoligams), and Ferulate (class of carbohydrate) respectively [16-18]. Most of the phytochemicals identified fall under alkaloids, flavonoids, and alkaloids from the leaf extracts of *Cyclea peltata*.

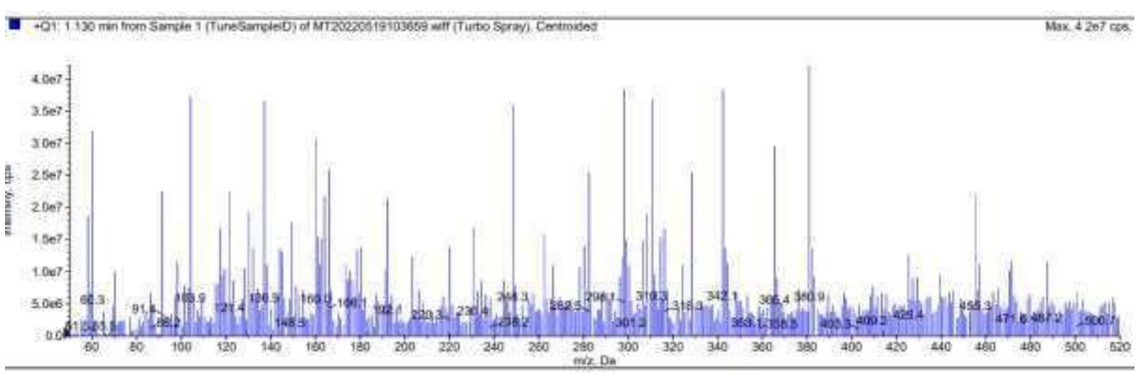

Figure 6: LC/MS-MS analysis of *Cyclea peltata*

DISCUSSION

The past of medicinal plants goes all the way to the earliest days of human existence, where plants acted as an essential source of food, shelter, and medicine. Over centuries, societies, especially tribal populations, have built a large treasure of traditional knowledge on the medicinal properties of plants. The majority of this information is still unrecorded, and few plant species have been scientifically investigated for their pharmacological activity. India, with its rich biodiversity and cultural wealth, is a key resource for medicinal plants, particularly in areas such as the Western Ghats. Traditional medicine has become a vital resource for contemporary drug discovery over the years, with the potential to provide leads for novel antimicrobial, anti-inflammatory, anti-diabetic, and anticancer agents. Medicinal plants are not only prized for their efficacy but also for safety, economy, and biocompatibility relative to their synthetic counterparts [19]. A large part of medicinal activity from plants comes from their antioxidant capacity. Phenolic compounds, commonly found in plant extracts, are primarily responsible for such activity. These natural antioxidants are important in scavenging free radicals, thus preventing oxidative stress-related diseases like diabetes, cardiovascular diseases, neurodegeneration, and cancer [20]. While synthetic antioxidants are available, natural antioxidants are preferred because they are multi-functional and less toxic. In addition, the application of medicinal plants is not limited to human medicine but also in veterinary medicine, especially in rural areas where herbal medicines are still a major source of healthcare for livestock. This not only serves to safeguard indigenous knowledge but also provides safer, more affordable substitutes for contemporary veterinary medicines, which tend to have residues and resistance risks [21-22].The antimicrobial activity of medicinal plants has been receiving growing attention, particularly against the backdrop of increasing antibiotic resistance. Numerous plants contain strong antibacterial, antifungal, and antiviral activities due to their diverse secondary metabolites such as alkaloids, flavonoids, and tannins [23]. As resistant pathogens challenge traditional therapies, plants offer a promising pathway to novel antimicrobial agents. The journey of utilizing these bioactive compounds starts with the judicious choice, identification, and harvesting of the plant material, considering such aspects as geographic location, season, and climate [24]. The follow-up processing activities of cleaning, drying, and extraction are essential in order to maintain the integrity and activity of the phytochemicals [25]. Different traditional and conventional extraction techniques permit the isolation and characterization of the compounds, paving the way to new therapeutic drugs [26].

In comparison with other research, the prominence of medicinal plants continually arises from different fields of study. All over the world, research endeavours are constantly confirming the potential of plant compounds in combating contemporary health conditions. Just as research has established the protective properties of polyphenols against oxidative stress or emphasized the powerful antimicrobial activity of ancient herbs, this corpus of work affirms the importance of ethnobotanical knowledge. The continued investigation and scientific validation of medicinal plants not only closes the gap between ancient practices and contemporary medicine but also underscores the imperative to preserve biodiversity and cultural heritage for the future [27-36].

2. CONCLUSION

The current research underscores the enormous antimicrobial potential of *Cyclea peltata* methanol extract due to the presence of alkaloids, flavonoids, and phenolic compounds in great proportion, detected by HPLC and LC/MS-MS analysis. The results uphold that the plants, which have been used in traditional folk medicine, contain useful bioactive components effective in fighting both gram-positive and gram-negative pathogens. The ethnomedicinal survey in the Vythiri Taluk, Wayanad district, also highlights the massive biodiversity and richness in medicinal plant resources in the Western Ghats region, which is of critical importance in supporting indigenous healthcare practices. Despite the encouraging results, the research also emphasizes the immediate necessity for intensive pharmacological and toxicological studies to establish the safety, efficacy, and mechanisms of action of these extracts. Furthermore, the accelerated loss of medicinally valuable species due to anthropogenic stresses necessitates instant conservation measures to safeguard these precious natural resources. Ethnographic research must continue to record traditional knowledge prior to its loss, providing new leads for drug discovery and the creation of new medicines. Therefore, this publication not only presents a scientific rationale for traditional practice but also provides a solid platform for research in the future to develop safe, plant-derived medicines.

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