Preliminary Phytochemical Analysis And AntimicrobialActivity Of Leaves Of *Hydrocotyle Verticillata* Thunb.

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Abstract:
Hydrocotyle verticillata Thunb. is a creeping medicinal plant with medicinal characteristics that grows mostly in gloomy and wet places. It is a member of the Araliaceae family. The purpose of this study was to look into the phytochemical screening and antimicrobial activity of *Hydrocotyle verticillata*. The leaf samples were gathered, dried in the shade, and pulverized. The extraction solvents were chloroform, methanol, hexane, ethyl acetate, and aqueous. The phytochemical constituents were found to be rich in flavonoids, phenol, and terpenoids. The antibacterialactivity of the leaf extracts were assessed against several common pathogenic bacteria and fungi usingthe agar disc diffusion method in order to assess the scientific foundation for the usage of the plant. Gram positive bacteria like *staphylococcus aureus*, *Enterococcus faecalis, Lactobacillus acidophilus* and *Bacillus subtilis,* Gram negative bacteria like *Klebsiella pneumoniae*, *Enterobacter aerogenes, Escherichia coli, Proteus vulgaris,* and fungi such as*Aspergillus niger, Aspergillus flavus, Penicillium notatum* were used and the width of the inhibition zone against the aforementioned pathogens provided as a measure of the concentrated extracts' antimicrobial activity. All extracts, with the exception of aqueous extracts, had excellent antifungal activity. Chloroform and ethyl acetate extracts were very effective against both gram positive and gram negative bacteria. The antibacterial action was brought on by phytochemicals such flavonoids, phenol, andterpenoids. The current study suggests that *Hydrocotyle verticillata* can replace synthetic drugs that are commercially accessible but may have a multitude of adverse effects as a potent source of natural antimicrobial agents.

Keywords: Araliaceae, Hexane, *Hydrocotyle verticillata*, Phytochemicals and Synthetic drug.

1. Introduction

Phytochemicals found in plants provide a wide range of medicinal benefits. Many chronic degenerative illnesses are protected from by phytochemicals found in medicinal plants [1]. Chemical substances that are naturally present in plants are known as phytochemicals. They are in charge of the plant's colour and organoleptic characteristics [2]. It is also known as those substances that have not been proven to be required nutrients for plants but may have biological importance [3].

Although phytochemicals might be obtained as dietary supplements, their potential health advantages come from eating the entire plant [4]. Numerous phytochemicals have a variety of functions that contribute to long-term disease immunity. It is generally recognised that some phytochemicals, such as alkaloids, tannins, flavonoides, carbohydrates, saponins, glycosides, phenols, phytosterols, diterpenes, amino acids, and protein among others, have both physiological and therapeutic properties [5,6]. Several plants have the potential to be used as medication sources, according to recent research [7]. Higher plants are a possible source of antibiotic prototypes, according to research evaluating plant extracts and natural sources for antimicrobial activity [8]. Numerous investigations have found various substances in medicinal plants that function as antibiotics [9]. Since 1926, scientistshave studied in labs the properties of plants that prevent germs and are significant for human health. A key source of novel antibiotic discoveries is the use of herbal treatments in traditional medical systems

across the globe [10]. Compounds from several conventional treatments have previously been discovered to be efficient against bacterial strains resistant to antibiotics [11].

Hydrocotyle verticillata Thunb. is commonly known as water pennywort, which comes under Araliaceae family. It is of cosmopolitan origin and common habitats of the plant are South and North America and West Indies. Its juice is used for the treatment of fevers. The poultice is used for the treatment of wounds. The decoction of the plant is applied for the treatment of influenza, abscesses, coughs, colds, hepatitis, purities, and sore throat. *Hydrocotyle verticillata* is used for urinary problems and headaches. It is given to kids in Malaya as a cough remedy after being combined with sugar and cassia bark. Its juice is considered emetic. For the purpose of treating skin conditions on the scrotum, plant leaves are mashed with alum. In China, the plant is used for the treatment of hepatoma and hepatitis.

Additionally, the plant is a component of Chinese herbal remedies for muscular dystrophy. Typhoid fever is treated in the Indian state of Arunachal Pradesh using the plant's juice combined with honey. In some parts of India, the juice is taken twice daily doses of three tablespoons for 5 days. Studies have shown effectiveness for antitumor, immune-modulatory, antioxidant, antiproliferative, new phytochemicals and saponins, and the presence of compounds that have benefits for the liver [12]. According to the review, very few works have been done in *Hydrocotyle verticillata*. The current study aims to assess the phytochemical analysis and antimicrobial activity of *Hydrocotyle verticillata* leaves.

2. Materials and Methods

2.1. Collection of plants

Hydrocotyle verticillata were collected from Melpuram, Kanyakumari district. To get rid of additional unwanted debris, the gathered leaves were washed in water. The leaves were shade-dried and powdered for further analysis.

2.2. Sample extraction

Soxhlet extraction was used to create crude plant extract. After the setup of Soxhlet apparatus using clamps and mounts. The round bottom flask is filled with solvent based on the nature of solvent (non-polar to polar = Hexane > Methanol > Chloroform > Ethyl acetate > Aqueous), around 250-300 ml of solvent is filled in the round bottom flask. Cotton is used to cover the hole of siphon or capillary tube in need to avoid the powdered sample to get settled in round bottom flask and also in siphon tube. The powdered sample were rolled in a cotton cloth and placed in the soxhlet thimble. Once the condenser is filled with running tap water. Isomantle (heat source) is used to evaporate the solvent; the temperature was fixed about the heating point of desired solvents. Repeatedly fifteen times the extraction is carried out by running the soxhlet apparatus. The extract was collected and used for further analysis.

2.3. Phytochemical analysis

For the analysis of qualitative and quantitative tests standard phytochemical assays were being carried out to determine the composition of the various extracts [13 to 19]. The phytochemical tests were analysed in carbohydrates, protein, alkaloids, flavonoids, glycoside, terpenoid, steroid, phenol, tannin and saponin.

2.4. Antibacterial Activity

2.4.1. Test Organisms

The test bacteria used in antimicrobial analysis such as *Staphylococcus aureus, Lactobacillus acidophilus, Bacillus subtilis, Enterococcus faecalis, E coli, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus vulgaris, Aspergillus niger, Aspergillus flavus,*and *Penicillium notatom* were bought from the Chandigarh-based MTCC (Microbial Type Culture Collection and Gene Bank). The bacterial strains are kept on Sabouraud Dextrose Agar, whereas the fugal strains were kept on Nutrient Agar (NA) (SDA).

2.4.2. Preparation of culture

Pure cultures were transferred from the plate to nutrient agar plates and subcultured for 24 hours at 37°C. The new culture was aseptic conditions added to 2 ml of sterile 0.145 mol/L saline tube to create the inoculum, and

the cell density was then corrected to the 0.5 McFarl and turbidity standard to produce a bacterial suspension with 1.5 108 cfu/ml. Antimicrobial test inoculum seed that is uniform [20].

2.4.3. Antibacterial Test

The medium was made by dissolving 38 g of Mueller-Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was placed in an autoclave for 15 minutes at 121°C and 15 lb of pressure (pH 7.3). After cooling and being thoroughly mixed, the autoclaved media was put into Petri plates (25 ml each). The pathogenic bacteria culture was used to swab plate's *viz*. *Staphylococcus aureus, Lactobacillus acidophilus, Bacillus subtilis, Enterococcus faecalis, E. coli, Klebsiella pneumoniae, Enterobacter aerogenes* and *Proteus vulgaris*. After that, the Sample or Sample loaded disc was put down on top of the Mueller-Hinton Agar medium. Empty sterile discs were utilised as a negative control, and a conventional medication disc containing 30 mcg of amikacin was used as a positive control.

For incubation, the plates were kept at 37°C for 24 hours. The inhibition zones surrounding the disc were examined after incubation and measured using a clean ruler in millimetres. The disc's size and the zone of inhibition's size were both measured in millimetres. It was assumed that there was no activity since there was no zone inhibition [21, 22].

Activities are expressed as resistant when the zone of inhibition is less than 7 mm, followed by moderate (8–10 mm) and sensitive (more than 11 mm) [23].

2.5. Antifungal Test

Agar disc diffusion technique [24] was used to determine the antibiotic susceptibility testing. Fungal strains such as *Aspergillus niger, Aspergillus flavus, Penicillium notatum* were swabbed on an SDA agar plate by sterilised cotton swabs. Up to 80 µl of each concentration of the extract was applied to the sterile discs using sterile pipettes (10 mm). The sterile disc (10 mm) was prefilled with the usual medication Fluconazole 150 mcg concentration as a positive control, and left empty as a negative control. Following the disc's placement on the SDA medium, the compound was given five minutes to diffuse before the plates underwent a 48 hours incubation period at 22°C. The inhibition zones surrounding the disc were examined after incubation and measured using a transparent ruler in millimetres.

3. Results

According to the current study, the *Hydrocotyle verticillata* plant exhibits the existence of many phytochemical components, including alkaloids, flavonoids, tannins, terpenoids, glycosides, phenols, steroids, proteins, and carbohydrates, as indicated in Table 1.

A number of microorganisms, including *Staphylococcus aureus, Lactobacillus acidophilus, Bacillus substilis, Enterococcus faecalis, E. coli, Klebsiella pneumonia, Enterobacter aerogenes,* and *Proteus vulgaris* were resistant to the antibacterial activity of *Hydrocotyle verticillata*. *Lactobacillus acidophilus* and *Proteus vulgaris* were the two bacteria that the extract of chloroform and ethyl acetate was most effective against. The highest activity of the methanol extract was against *Proteus vulgaris* and *Bacillus substilis*. According to Table 2, the aqueous extract has greatest effectiveness against *E. coli* and *Staphylococcus aureus*. The majority of the studied microorganisms exhibit no resistance to hexane extract. The common antibiotic *Amikacin* was utilized as a positive control for different extracts, while a sterile water disc was used as a negative control.

By evaluating the width of the zone of inhibition, the antifungal activity was assessed. The *Hydrocotyle verticillata* extracts with the highest antifungal activity against *Aspergillus flavus* were determined to be ethyl acetate and methanol extracts. All of the *Hydrocotyle verticillata* extracts that were examined shown antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium notatum*; however, an aqueous extract failed to demonstrate antifungal activity against *Aspergillus niger* and *Pencillium notatum* (Table 3). Fluconazole, a common antibiotic, was employed as a positive control.

Table1. Phytochemical analysis of *Hydrocotyle verticillata* with different solvents

Phytochemical constituent	Hexane	Chloroform	Methanol	Ethyl acetate	Aqueous
Alkaloid	-	-	+	-	+
Flavonoid	+	+	+	+	+
Tannin	+	-	+	-	+
Saponin	-	-	-	-	+
Terpenoids	+	+	-	+	+
Glycoside	-	+	+	+	+
Phenol	+	+	+	+	+
Steroids	_	-	+	-	+
Proteins	-	-	+	-	+
Carbohydrate	-	+	-	+	+

+: present, - absent

Table 2. Antibacterial activity of *Hydrocotyle verticillata*

Bacterialstrains	Extracts (mm)					+ve Control	-ve control
	Hexane	Methanol	Chloroform	Ethyl acetate	Aqueous		
Staphylococcus aureus	NZ	8	7	7	10	25	NZ
Lactobacillus acidophilus	9	9	16	15	NZ	24	NZ
Bacillus substilis	NZ	12	7	7	7	20	NZ
Enterococcus faecalis	NZ	NZ	9	8	11	21	NZ
E.coli	NZ	7	7	7	13	19	NZ
Klebsilla pneumonia	NZ	7	9	8	8	20	NZ
Enterobacter aerogenes	NZ	7	8	8	9	20	NZ
Proteus vulgaris	7	12	13	13	9	17	NZ

+ve Control – Amikacin; -ve Control – Sterile water; NZ- No Zone of Inhibition; mm- millimeter

Table 3. Antifungal activity of leaves of *Hydrocotyle verticillata*

Fungal strains	Extracts (mm)					+ve control	-ve control
	Hexane	Methanol	Chloroform	Ethyl acetate	Aqueous		
Aspergillusniger	9	10	7	9	NZ	16	NZ
Aspergillus flavus	8	13	7	14	10	25	NZ
Penicillium notatum	9	7	10	10	NZ	21	NZ

+ve Control – Fluconazole; -ve Control – Sterile disc water; NZ- No Zone of Inhibition; mm-millimeter flavonoids and their target locations, might lead to

4. Discussion

Plant materials have been used in phytomedicine since the dawn of mankind. The fact that they may be derived from any part of the plant suggests that all of the plant's components may contain active chemicals. Although it will be helpful in the synthesis of complex chemical compounds, knowledge of plant chemical components is essential. Numerous studies on the phytochemical screening of various plants have been published [25,26]. The majority of the chosen

plants included coumarins, glycosides, flavonoids, saponins, phenols, tannins, and steroids which might be the cause of the observed antibacterial property [27]. Plants high in flavonoids and tannins have been shown to have antibacterial properties [28]. By inactivating bacterial enzymes, this is done.

Furthermore, secondary metabolites including tannins and other phenolic compounds are classed as active antibacterial compounds [29]. Further research into the mechanism of action as well as the interaction between these active the creation of new pharmacological molecules with antibacterial activity [30]. The presence of these active phytochemicals in the plant extract *H.verticillata* suggests that the presence of these secondary plant products may aid in the clarification of their distinct antibacterial properties.

Saponin's antibacterial activity is due to its potential to cause protein and enzyme leaks from the cell. The link between membrane lipids and susceptibility to steroidal substances demonstrates that steroids selectively interact with membrane lipids and exert their effect by generating leakages from liposomes [31], despite the fact that steroids were reported to have bactericidal capabilities. Antibacterial effects of plant-derived phenolic compounds have been demonstrated [32]. Polyphenols and sterols have been found to inhibit bacterial and fungal growth. Leaf extracts in Hexane, Methanol, Chloroform, Ethyl acetate, and Aqueous were employed in the current *in vitro* study. The current investigation found that chloroform and methanol had the greatest inhibitory action, whereas ethyl acetate has the most antimicrobial activity.

5. Conclusion

The results showed that the *H. verticillata* leaves exhibited a range of antimicrobial effects on the selected microbes. Our findings imply that *H. verticillata* extracts contain phytocompounds with potent antimicrobial characteristics that can be exploited as antimicrobial agents in the search for novel medications and promote the use of the plant as a traditional medicine. To extract, define, identify, and understand the structure of the bioactive components, more research is ongoing.

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