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Centella Asiatica: Phytochemical extraction with bio Solvents and Phytochemical Screening

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Abstract
Medicinal plants are the conventional sources of phyto therapeutic substances that are used to treat various diseases since pre-historic times. Centella asiatica is one of the ancient therapeutic herbs known for its traditional use worldwide. The presence of phytochemicals imparts the pharmacological value to the medicinal plants and hence their extraction is significantly important. Many of the works include extraction of phytochemicals by hazardous VOCs. The present work explored the phytochemical extracting ability of three bio solvents ethyl lactate, ethanol and hydroalcohol from Centella asiatica. Qualitative phytochemical screening of these extracts was carried out and summarized.

Introduction

Medicinal plants are the important sources of pharmacological compounds used by many pharmaceutical companies to prepare different formulations. Use of herbal products for therapeutic needs has become a regular practice worldwide. As per World health organization (WHO) reports, 80% of the world's population depends on the herbal medicines [1]. Medicinal plants have become unique sources of medicines and may constitute the most common human use of biodiversity. They are the richest bio-resource of traditional systems of medicine, modern medicines, folk medicines, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs [2].

Herbal medicines are simple and possess broad range of activity with minimum side effects and hence considered advantageous over the other class of drugs or chemotherapeutic drugs. Since ancient times, these have been the part of traditional medicinal systems like Ayurveda, Unani and Siddha. There are approximately 426 biomes existing in the world that are richest plant genetic resources [3]. About 3000 plants out of 18,665 flowering species have been in use for the preparation of therapeutic drugs in the traditional systems of medicine [4].

Centella asiatica is one of the important herbaceous perennial plant that grows in the tropical and sub-tropical regions like India, Bengladesh and Sri Lanka. It belongs to the flowering plant family 'Apiaceae' and it's common names include Gotu Kola, Indian Pennywort and Mandookarparni. It forms a dense green carpet on moist, sandy or clayey soils and grows up to the height of 600 to 800m above the sea level [5,6].

The characteristic features of the plant are simple, long arachnoid petioled leaves on a creeping stalk with 2-4 flowered pink blossoms, rooted at the nodes, obsolete Calyx teeth Short, purple, depressed stylopodium; styles Fruit is 2-3 mm long, oblong to orbicular, and has primary and secondary ridges that create a network. The vittae are not clearly defined. [7 - 9].

Specific bioactive chemical compounds of the medicinal plants exhibit physiological action on human body. The medicinal properties of the plants are mainly due to the presence of bioactive secondary metabolites like alkaloids, cardiac glycosides, phenols, tannins, flavonoids, sterols, saponins, reducing compounds, mineral and vitamins [10,11].

Centella asiatica contains several triterpenes, saponins like asiaticoside, Asiatic aid, sapogenins, madecassic acid, vellarin, adecassoside, glycosides and

centellosides [12]. It is used as a medicinal herb in Ayurvedic medicine, Western herbal medicine, traditional African medicine, traditional Chinese medicine and in Western orthodox medicine, for example to stimulate the regeneration of skin burns while preventing the formation of scar tissue. It is one of the important medicinal plants in the international market of medicinal plant Trade and is used in the world for different purposes and diseases.

Fig.1: Centella asiatica leaves and powder

Scientific classification

CLASS	Dicotyledenae
SUB CLASS	Polypetalae
SERIES	Calyciflorae
ORDER	Umbellales
FAMILY	Umbelliferae(Apiaceae)
GENUS	Centella
SPECIES	asiatica

Medicinal uses

In India, China, and Indonesia, Centella asiatica has been used for thousands of years to cure a variety of ailments. It was used to treat skin disorders including leprosy and psoriasis, heal wounds, and enhance mental clarity. Additionally, it is one among the ingredients in the popular summertime beverage is known as “thandaayee” [13]. In addition, Malay and Japanese people frequently consume centella as a green leafy vegetable in salad and ulam [14]. The salad doubles as an appetiser because it is rich in micronutrients. You can also make soup with it. Due to the presence of Vellarine, it has a bitter flavour and is typically eaten with coconut milk or sweet potatoes.

Centella asiatica leaves are combined and utilised in Thailand to make tea, juice, and cordial drinks [15, 16]. To counteract malnutrition, the leaves are utilised in Sri Lanka to make the traditional curry "mallung" and the porridge "kolakenda" [17]. Food items like herbal noodles are made using extract from the Centella asiatica plant [18]. It is utilised in China as a cooling beverage [19, 20].

Phytochemicals

Plants produce phytochemical to sustain and protect themselves from environmental factors. High concentrations of phytochemicals may accumulate in fruits and vegetables and protect them against free radical damage [21]. Most of the plants possess antioxidants as one of the phytochemicals. They can By postponing or preventing oxidation brought on by reactive oxygen species, you can regulate and limit the oxidative damage to foods (ROS). As a result, shelf-life and quality of the food increases [22]. Many phenolic compounds, ascorbic acid, beta carotene etc. play active role in age delay, inflammation reduction, and cancer prevention [23]. The following phytochemicals are considered for study and their importance are discussed here.

Alkaloids

some related substances that have neutral [24] or even mildly acidic characteristics [25]. Alkaloids can also contain oxygen, sulphur, and, less frequently, additional elements like chlorine, bromine, and phosphorus in addition to carbon, hydrogen, and nitrogen [26]. Alkaloids exhibit a wide variety of pharmacological effects, such as antimalarial (Quinine), antiasthmatic (Ephedrine), anticancer (Homoharringtonine), cholinomimetic (Galantamine), vasodilatory (Vincamine), analgesic (Morphine), and antibacterial (Chelerythrine) [27, 28, 29, 30].

Phytosterols/ steroids

Phytosterols are a class of naturally occurring substances that are present in the membranes of plants. They are also known as plant sterols and stanol esters. Due to their structural resemblance to the body's cholesterol, phytosterols compete with cholesterol for absorption in the digestive system when eaten. As a result, blood cholesterol levels are decreased and cholesterol absorption is stopped [31].

The Food and Drug Administration (FDA) has even approved a health claim on phytosterols, which states: "Foods containing at least 0.65 gram per serving of vegetable oil plant sterol esters, eaten twice a day with meals for a daily total intake of at least 1.3 grams, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease."

Unrefined vegetable oils, whole grains, nuts, seeds, and legumes are excellent sources of phytosterols. Phytosterols could enhance serum lipid (cholesterol) profiles and lower the risk of cardiovascular disease by being added to the current diet [32].

The sterol ring of plant sterols has a double bond. The three sterols stigmasterol, campesterol, and -sitosterol are the most prevalent in both plants and the human diet. In the sterol ring, plant stanols lack a double bond. Only around 10% of all dietary phytosterols are stanols, particularly sitostanol and campestanol.

Carbohydrates

Compounds created during photosynthesis are known as carbohydrates. They provide two key functions in plants. They first give plants the building blocks for their structural parts, and then they give plants the energy they need to thrive. Among the group of carbohydrates are cellulose, starches, and sugars. Energy for cellular processes is provided by sugars and starches, and the fibrous material cellulose makes up the cell wall of plants.

Glycosides

Glycosides are a class of naturally occurring chemicals that have been studied for their potential antibacterial, antifungal, anti-inflammatory, antioxidant, antiviral, and anticancer properties. They are present in many different plant groups. Anthraquinone, cardiac, chromone, coumarin, cyanogenic, flavonoid, saponin, steroidal, and steviol glycosides are a few of the more than 11 notable classes[33].

Saponins

Many plants contain saponins, which are glycosides with a peculiar foaming property. The name comes from the plant soapwort, whose root was previously used to make soap [34]. The saponins' capacity to foam is due to the interaction between the nonpolar sapogenin and the water-soluble side chain. They are harsh and make animal meals less palatable. While others are not very detrimental, some saponins decrease the feed intake and development rate of nonruminant animals.

Tannins

Plant tannins are a broad, diversified category of polyphenolic chemicals that are present in many different plant species. Tannins provide a protective role in the bark of a plant's roots, stems, or other exterior layers. They have a high polyphenol content, which gives them an astringent quality. Strong complexes can be formed with proteins, carbohydrates, and other macromolecules thanks to this quality [35].

Flavonoids

A class of plant metabolites known as flavonoids is hypothesised to have positive impacts on health via activating antioxidant and cell signalling pathways. Numerous fruits and vegetables contain these compounds [36].

Chalcones, flavones, isoflavonoids, flavanones, anthoxanthins, and anthocyanins are the different types of flavonoids. Numerous of these molecules, in particular the anthoxanthins, give petals a yellow tint, whilst the anthocyanins are frequently responsible for the red colour of buds and the purple-red hue of fall leaves.

Flavonoids are safe for animals, including humans, to consume in high quantities due to their abundance and low toxicity. Flavonoids are found in foods like onions, parsley, blueberries, bananas, dark chocolate, and red wine, to name a few.

Phlobatannins

Tannins that yield phlobaphenes with hot dilute acids are called phlobatannins [37]. These are phenolic compounds that are crimson, alcohol-soluble, and water-

insoluble. They can be obtained by extracting tannins from plants or by treating tannin extracts with mineral acids. [38]

Anthraquinone

An significant subfamily of the quinone family, anthraquinones include a wide range of structurally different compounds in the polyketide family. Typically, they take on their glycosidic forms for example, the sennosides from senna (*Cassia species*). These substances give plants their colour and are frequently used as natural dyes. They also function as laxatives and have antifungal and antiviral properties [39].

Resins

Plant resins are primarily a combination of terpenoid and/or phenolic chemicals that are lipid-soluble and both volatile and nonvolatile. These are typically hidden in specialised organs that are either internal or external to the plant. The secondary metabolites that make up the resins have no function in the plant's core or fundamental physiology.

Phenols

Phenols from plant origin are of three types.
(i) Phenolic acids, (ii) Flavonoids, and (iii) Nonflavonoid polyphenols. Phytochemical phenols possess definitive roles in plants as the ultraviolet (UV) protectants, wound-healing action, disease and pest resistance.
Plant phenols include compounds like flavonoids, cinnamic acids, benzoic acids, stilbenes, proanthocyanidins, coumarins, lignans and lignins. They are potent antioxidants and prevent oxidative damage to biomolecules such as DNA, lipids and proteins which play a role in chronic diseases such as cancer and cardiovascular disease [40].

Literature Review

Due to its extensive positive neuroprotective activity, centella asiatica has been regarded as a brain tonic. Along with this, a number of additional benefits have been noted, including anti-inflammatory, antiproliferative, anticancer, antioxidant, antiulcer, wound healing, etc. Numerous phytochemicals, including polyphenolic compounds, asiatosides, and asiatic acid, have been shown to be useful for these effects when these effects were evaluated on animal models. The plant is also examined for its toxicity and potential medication interactions. Centella asiatica was discovered to interact with anticonvulsant medications, and a taxological research advised using it for an extended length of time with caution. Additionally, there are several commercial goods out there that have primarily been used for dietary supplements, skin nourishment, antioxidants, and memory enhancement[41].

Wide-ranging information on the present research aspects of the herb and it's pharmacology, mechanisms of functioning, preclinical and clinical studies are reviewed [42].

Medicinal uses of Centella asiatica were described for treating skin problems, revitalizing the nerves, wound healing, as a brain tonic, and many other applications [43].
Comprehensive neuroprotection action of Centella asiatica has been reported by different modes of action such as prevention of amyloid plaque formation in Alzheimer's disease, enzyme inhibition, and decreasing oxidative stress [44].

Phytochemical study of Centella asiatica has been reported based on various physiochemical characteristics as water and alcohol solubility; succeeding extractive values (pet.Ether, chloroform, acetone, alcoholic, aqueous), moisture level, total proportion of ash, Bulk density, medication pH in 1% and 10% concentrations, and weight loss during drying [45].

Reported the dried powdered leaves of C. asiatica (L) were extracted using four different solvents, including water, acetone, chloroform, and methanol [46].

Aim and scope of the present work

Many of the works include extraction of phytochemicals by hazardous VOCs. As per the green context and sustainable environment, bio compounds as extracting solvents could be suggestible. As per authors knowledge, very little work is available in this regard. So, there is a need to study the extraction of

phytochemicals by bio solvents.

The present work aims to explore the bio solvents ethyl lactate, ethanol and hydroalcohol for their phytochemical extracting ability from *Centella asiatica* and to carry out the qualitative phytochemical screening of these extracts.

Materials and Methods

Centella asiatica is chosen for its phytochemical extraction with bio solvents and the extracts were tested for the presence of phytochemicals.

Plant material collection

Centella asiatica leaves were collected from the college campus (Maris Stella college, Vijayawada, A.P., India), washed, shade dried and powdered.

Preparation of Plant Extract

Extraction method: Maceration

Extracting solvents: Ethyl lactate, Ethanol and Hydro alcohol. All the three are green solvents with good solvent power. They are benign, 100% biodegradable and bio based. They provide safer solvent exposure. Based on green context, their extracting abilities were considered for study.

Centella asiatica leaf extract is prepared by cold extraction method 'Maceration'. 50g of powdered plant material is mixed with 100ml of solvent and is taken in a stoppered conical flask. The flask is kept on an orbital shaker (Pharmacon company) for continuous agitation for a period of 5 days until the soluble matter has dissolved. The contents are filtered through vacuum filtration and stored in refrigerator until further usage.

Phytochemical screening

The leaf powder of Centella asiatica is extracted with solvents ethyl lactate, ethanol and hydroalcohol to screen the following phytochemicals by using standard qualitative methods [47].

Tests for alkaloids

Plant extract is shaken well with dilute HCl and filtered. The filtrate is used for the following tests

a) **With Mayer's reagent:** A little extract is mixed with few drops of thereagent and shaken well. Formation of pale yellow precipitate indicates the presence of alkaloids.

b) **With Wagner's reagent:** A little extract is mixed with few drops of the reagent and shaken well. Formation of reddish brown precipitate indicates the presence of alkaloids.

c) **With Hager's reagent:** A little extract is mixed with aqueous saturated solution of picric acid and shaken well. It is then heated and cooled under tap water. Formation of yellow shiny precipitate indicates the presence of alkaloids.

d) **With 5% Tannic acid reagent:** A little extract is mixed with 1ml of the reagent and shaken well. Formation of buff coloured precipitate indicates the presence of alkaloids.

Tests for steroids

a) Salkowski's Test: A little extracts is treated with chloroform and few drops of Conc. Sulphuric acid is added along the sides of the test tube and allowed to stand. Appearance of reddish-brown ring at the interface indicates the presence of steroids.

b) Liebermann Burchard test: A little extracts is mixed with chloroform and acetic anhydride. Then few drops of Conc. Sulphuric acid is added along the sides of the test tube and allowed to stand. Appearance of green colour indicates the presence of steroids.

Tests for Carbohydrates

A little extract is shaken well with distilled water, filtered and used for the following tests.

a) Molisch's Test: A little Filtrate is mixed with 2 drops of alcoholic α-naphthol solution in a test tube. Then few drops of Conc. Sulphuric acid is added along the sides of the test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's test: Filtrate is treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's Test: Filtrate is hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

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Tests for Glycosides

Extract is hydrolyzed with Conc HCl for 2 hrs on a water bath, filtered and the filtrate is subjected to the following tests.

a) Borntrager's Test: A little extract is shaken well with chloroform and allowed to settle. Organic layer is separated and 10% ammonia solution is added to it. Pink colour indicates the presence of glycosides.

b) Legal's Test: A little extract is dissolved in pyridine. To this sodium nitroprisside solution is added and made alkaline with 10% NaOH. Pink colour indicates the presence of glycosides.

Test for Saponins

Extract is diluted with about 20ml distilled water taken in a stoppered conical flask and shaken well for 10 min. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for Tannins

A little extract is shaken with distilled water andfiltered. To the filtrate neutral $FeCl_3$ is added and shaken well. Appearance of green colour indicates the presence of tannins.

Test for Flavanoids

A little amount of magnesium powder and few drops of Conc HCl are addedto 2 ml of the extract. A red or intense red colouration indicated the presence of flavanoids.

Test for Phlobatannins

A little extract is dissolved in distilled water and filtered. The filtrate is boiled with 2% HCl solution. Red precipitate shows the presence of Phlobatannins.

Test for Anthraquinones

A little extract is shaken with 4 ml of benzene. The mixture is filtered and 2 ml of 10% ammonia solution is added to the filtrate and shaken well. Appearance of pink, red or violet colour in the ammoniacal (Lower) phase indicated the presence of free anthraquinones.

Test for Resins

1:1 copper acetate solution and extract are taken in a test tube and shaken vigorously, allowed to separate. A green coloured solution indicates the presence of resin.

Results and Discussion

Phytochemical Screening

The preliminary phytochemical screening revealed the presence of alkaloids, steroids, carbohydrates, glycosides, saponins, tannins, flavanoids, anthraquinones, resins and phenols, in some of the extracts (ethyl lactate, ethanol, hydroalcohol) as shown in the Table 1.

S.No	Phytochemical	Extracting solvent		
		Ethyl lactate	Ethanol	Hydroalcohol
1	Alkaloids	+ ve	+ ve	+ ve
2	Steroids	+ ve	+ ve	+ ve
3	Carbohydrates	+ ve	+ ve	+ ve
4	Glycosides	- ve	+ ve	+ ve
5	Saponins	- ve	- ve	+ ve
6	Tannins	+ ve	+ ve	+ ve
7	Flavanoids	- ve	- ve	- ve
8	Phlobatannins	- ve	- ve	- ve
9	Anthraquinone	- ve	- ve	- ve
10	Resins	+ ve	+ ve	+ ve

11	Phenols	+ ve	+ ve	+ ve

Table 1: Phyto constituents of different extracts of Centella asiatica

54.5%, 64% and 82% of the tested phytochemicals are identified in ethyl lactate, ethanol and hydroalcohol extracts respectively. With all the three extracts, negative tests are observed for anthraquinone and phlobatannins. Flavanoids were extracted only with Hydroalcohol.

Conclusion

Centella asiatica leaf extracts were prepared by maceration method using bio solvents ethyl lactate, ethanol and hydroalcohol. The extracts were subjected to qualitative phytochemical analysis using standard procedures.

- More number of phytochemicals were extracted into hydroalcohol.
- Nine phytochemical compounds such as alkaloids, steroids, carbohydrates, glucosides, saponins, tannins, phenols, flavonoids and resins were found in the extracts.

The results suggest that the leaves of *Centella asiatica* are rich source of valuable primary and secondary metabolites.

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