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Study On The Phytochemical Screening Of Extracts From The Leaves Of Centella Asiatica

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ABSTRACT
Centella asiatica(L) commonly known as Asiatic pennywort which is having great medicinal history. It is a small herbaceous medicinal plant belongs to the family Apiaceae family. It is used as a traditional ayurvedic medicine. The active metabolites like Alkaloids, flavonoids, Glycosides, Saponins, Phenolic compounds etc which has wide range of pharmaceutical uses like Memory enhancing, Treatment of skin related diseases, Wound healing etc. Current study is focused on phytochemical screening of shade dried leaves of Centella asiatica by Soxhlet extraction.
Key words: Centella asiatica, phytochemicals, Soxhlet, polar and non- polar Solvents.

1. INTRODUCTION

Centella asiatica (L) commonly known as Indian Pennywort belongs to the family Apiaceae.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 in burns while preventing the formation of scar tissue. The medicinal properties of the plants are mainly due to the presence of secondary metabolites like alkaloids, cardiac glycosides, tannins, flavonoid, saponins, reducing compounds, minerals and vitamins.
Species Centella are small prostrate herbs rooting at the nodes, but mostly they are with stout hollow internodes. The plants usually have an aromatic smell due to the presence of essential oil or resin in all organs .The leaves are alternate, but they are palmately compound. The petiole is often swollen and sheathing at the base and stipules are absen Flowers are fascicled umbels, each umbel consisting of 3-4 white to purple or pink, sessile flowers. Fruits are schizocarp with globular shape of 4 mm long 2,3,4. It has dehiscent seed which has a hard oily endosperm and a small embryo.
Centella asiatica, a small edible, herbaceous medicinal plant native to India, Sri Lanka, Iran, New Guinea, Australia, Indonesia southern and central Africa. The plant is listed as an important drug in the Indian Herbal Pharmacopoeia, European Pharmacopoeia, Pharmacopoeia of the People's Republic of China and German Homeopathic Pharmacopoeia. In 2006, it was ranked third position in a priority list of most essential Indian medicinal plants based on their pharmaceutical and economic importance and also the demands for its raw material is constantly rising throughout the world. Centella contains several valuable secondary compounds or terpenoids, known as centelloids, which includes Asiaticoside, madecassoside, centelloside, centellose, brahminoside, thankunizide, brahmoside, and asiatic, centellic, brahmic, and madecassic acids.The most biologically active compounds include triterpene saponins like asiatic acid, Asiaticoside, madecassoside and madecassic acid, which are responsible for a wide range of therapeutic activity.Antioxidant is any substance which prevents or retards deterioration richly available in fruits and green leafy vegetables which is known to reduce the risk of cancer, cardiovascular diseases. Some antioxidant components are ascorbic acid, carotenoids, vitamin E, polyphenols and other phytochemicals. A free radical is substance with unpaired electrons causes damage to lipids, protein, enzyme thus antioxidants prevents these damages by neutralizing them. Phytochemical imparts health benefits beyond basic nutrition. Several plant extracts and class of phytochemicals are known to have

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antioxidant activity. Flavonoid are potent antioxidant and its capacity depends on their molecular structure and Quercetin is one the most abundant dietary flavinoid. It was also reported that plant contains tannins, sugars, inorganic acids, amino acids like phenylalanine, aspartic acid, α alanine, glutamic acid and glycerin. The antioxidant activity of Centella plays a key role in acting against the reactive oxygen species in our body. Due Total flavonoid contents; Total tannins content.

Medicinal plants have been used all over the world as 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, food supplements.Under indigenous system of medicine like Ayurveda, Siddha and Unani. The plant is known as 'thankuni' in Bengali, 'Indian pennywort' in English, 'gotu kola' in Sinhala, 'mandukaparni' in Sanskrit (Ayurveda).

Phytochemicals are chemical compounds produced by plants, generally to help them resist fungi, bacteria and plant virus infections, and also consumption by insects and other animals. The name comes from Greek φυτόν *(phyton)* 'plant'. Some phytochemicals have been used as poisons and others as traditional medicine.

- As a term, *phytochemicals* is generally used to describe plant compounds that are under research with unestablished effects on health, and are not scientifically defined as essential nutrients. Regulatory agencies governing food labelling in Europe and the United States have provided guidance for industry to limit or prevent health claims about phytochemicals on food product or nutrition labels.
- Phytochemicals are chemicals of plant origin. Phytochemicals (from Greek *phyto*, meaning "plant") are chemicals produced by plants through primary or secondary metabolism. They generally have biological activity in the plant host and play a role in plant growth or defence against competitors, pathogens, or predators.
- Phytochemicals are generally regarded as research compounds rather than essential nutrients because proof of their possible health effects has not been established yet. Phytochemicals under research can be classified into major categories, such as carotenoids[6] and polyphenols, which include phenolic acids, flavonoids, stilbenes or lignans. Flavonoids can be further divided into groups based on their similar chemical structure, such as anthocyanins, flavones, flavanones, isoflavones, and flavanols.Flavonoid are further classified as catechins, epicatechins, and proanthocyanidins. In total, between 50,000and 130,000 phytochemicals have been discovered.
- Phyto chemists study phytochemicals by first extracting and isolating compounds from the origin plant, followed by defining their structure or testing in laboratory model systems, such as *in vitro* studies using cell lines or *in vivo* studies using laboratory animals. Challenges in that field include isolating specific compounds and determining their structures, which are often complex, and identifying what specific phytochemical is primarily responsible for any given biological activity.

The phytochemical category includes compounds recognized as essential nutrients, which are naturally contained in plants and are required for normal physiological functions, so must be obtained from the diet in humans.

- Some phytochemicals are known phytotoxins that are toxic to humans; for example aristolochic acid is carcinogenic at low doses. Some phytochemicals are antinutrients that interfere with the absorption of nutrients. Others, such as some polyphenols and flavonoids, may be pro-oxidants in high ingested amounts.
- Non-digestible dietary fibres from plant foods, often considered as a phytochemical, are now generally regarded as a nutrient group having approved health claims for reducing the risk of some types of cancer and coronary heart disease.
- Eating a diet high in fruits, vegetables, grains, legumes and plant-based beverages has long-term health benefits, but there is no evidence that taking dietary supplements of non-nutrient phytochemicals extracted from plants similarly benefits health. Phytochemical supplements are

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neither recommended by health authorities for improving health nor approved by regulatory agencies for health claims on product labels.

2. MATERIALS AND METHODOLOGY

2.1. MATERIALS:

2.1.1. SAMPLE:

Fresh leaves of Centella asiatica are collected and washed with a water and they are shade dried for 3 - 4 days to avoid the water content from the leaves and they sliced into small fine pieces.

2.1.2. CHEMICALS AND REAGENTS

- Sodium hydroxide (NaOH) (Hi LR)
- Hydrochloric acid (HCl)
- Glacial acetic acid
- Magnesium turnings
- Ethyl ether
- Sulphuric acid (H_2SO_4)
- Copper sulphate ($CuSO_4$)
- Ferric chloride ($FeCl_2$)
- Dragondroff's reagent

2.1.3. EQUIPMENT

- Beakers
- Petri dishes
- Conical flask
- Measuring cylinders
- Test tubes
- Pipes
- Electronic balance
- Soxhlet apparatus
- Heating mantle
- Soxhlet condenser
- Distillation unit

2.2. METHODOLOGY

2.2.1. SAMPLE PREPARATION:

Centella asiatica leaves are collected and cleaned in water to avoid the unwanted substances like mud and they are kept on moisture absorbing sheets like newspaper or blotting sheets and the are allowed for shade drying for 4 - 5 days to avoid water content from the leaves and after drying completely they are grinned or crushed in to the powder form and then the sample is filled in to the filter paper and it is placed in the thimble.



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2.2.2. PREPARATION OF TEST SOLUTIONS:

➢ **PREPARATION OF 40% NAOH SOLUTION:**
40 grams of NaOH Pellets is weighed and it is dissolved in a small amount of distilled water and the makeup up the solution to 100ml water.

➢ **PREPARATION OF 10% NAOH SOLUTION:**
10grams of NaOH is dissolved in small amount of distilled water and makeup it to 100ml by using measuring cylinder.

➢ **PREPARATION OF 1% COPPER SULPHATE SOLUTION:**
1g of copper sulphate is dissolved in small amount of distilled water and dissolve it and makeup the solution to 100ml in volumetric flask. 10% AQUACEOUS FERRIC CHLORIDE: 10g of sodium ferric chloride is dissolved in small amount of distilled water and dissolve it and makeup the solution to 100ml.

2.2.3. PHYTOCHEMICAL ANALYSIS:

➢ **TEST FOR ALKALOIDS-(Dragondroff's test):**
In a test tube containing 0.5 g of extract was defatted with 5%ethyl ether for 15 minutes. The defatted sample was extracted for 20 minutes with 5 ml of 1% aqueous Hcl on boiling water water bath and filtered to 1ml of filtrate add few drops of Dragondroff's reagent and the colour developed appearance of prominent yellow colour indicates the presence of alkaloids.
➢ **TEST FOR COUMARINS:**
To 1ml of extract 1ml of 10%NaOH was added the presence of coumarins is indicated by the formation of yellow colour.
➢ **TEST FOR STEROIDS (Liebbermann burchard test)**:
To 1ml extract 1ml of glacial acetic anhydride and 2 drops of conc h2so4 were added the solution becomes red then blue and finally bluish green indicated the of steroids.

➢ **TEST FOR SAPONINS:**
To extract 5 ml of water was added and tube was shaken vigorously. copious lather formation indicates the presence of saponins.

➢ **TEST FOR FLAVONES:**
To the extract a few magnesium turnings and 1-2 drops of conc Hcl were added formation of red colour shows the presence of flavones.

➢ **TEST FOR TANNINS:**
To the extract ferric chloride was added formation of a dark blue or greenish black colour shows the presence of tannins.

➢ **TEST FOR QUINONES:**
To 1ml extract 1ml of conc sulphuric acid was added formation of red colour shows the presence of quinones.

➢ **TEST FOR FLAVANONES:**
To the substance 10% NaOH was added yellow to orange colour shows the presence of flavanones. To the substance conc sulphuric acid was added orange to crimson red colour confirms the presence of flavanones.

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➢ **TEST FOR ANTHOCYANINS**:
To the substance 10% NaOH was added blue colour shows the presence of anthocyanins to the substance con sulphuric acid was added yellowish orange colour confirms the presence of anthocyanins.

➢ **TEST FOR PHENOLS**:
Ferric chloride test to the extract few drops of 10% aqueous ferric chloride was added appearance of blue or green colour indicates the presence of glycosides.

➢ **TEST FOR PROTEINS**:
To the extract 1ml 0f 40% NaoH solution and two drops of 1% cuso4 Solution were added formation of violet colour indicates the presence of proteins.

➢ **TEST FOR GLYCOSIDES**:
To 1ml of extract add 0.5 ml of glacial acetic acid 1 drop of 10% aqueous ferric chloride and 2-3 drops of conc sulphuric acid formation of brown ring at the interface indicates the presence of glycosides.

➢ **TEST FOR TERPENOIDS**:
2ml of extract was added to 2 ml of glacial anhydride and conc sulphuric acid formation of blue green rings indicate the presence of terpenoids.

3. RESULTS AND DISCUSSION:

The different phytochemical constituents present in the extract were identified by the colour reaction with different reagents. The main objective of phytochemicals screening was to identify the different groups of chemical constituents present in the plant extract. Results of the phytochemical screening are summarized below.

Phytochemical analysis of *Centella asiatica* in solvent petroleum ether

SL.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	Alkaloid test	Orange red colour formation	+
2.	Coumarins test	Yellow colour formation	+
3.	Steroid test	Red colour formation	-
4.	Saponins test	Copious lather formation	+
5.	Flavones test	Red colour formation	-
6.	Tannins test	Dark blue or greenish black	+
7.	Quinones test	Red colour formation	-
8.	Flavanones	Yellow to orange Orange to crimson red	-
9.	Anthocyanins	Blue colour yellowish orange	-
10.	Phenols	Blue or green colour	+
11.	Proteins	Violet formation	+
12.	Glycosides	Brown ring at the interface	-
13.	Terpenoids	Red brown colour	-

Phytochemical analysis of *Centella asiatica* in solvent Chloroform

SL.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	Alkaloid test	Orange red colour formation	+
2.	Coumarins test	Yellow colour formation	-

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3.	Steroid test	Red colour formation	**-**
4.	Saponins test	Copious lather formation	+
5.	Flavones test	Red colour formation	**-**
6.	Tannins test	Dark blue or greenish black	+
7.	Quinones test	Red colour formation	-
8.	Flavanones	Yellow to orange Orange to crimson red	-
9.	Anthocyanins	Blue colour yellowish orange	-
10.	Phenols	Blue or green colour	+
11.	Proteins	Violet formation	+
12.	Glycosides	Brown ring at the interface	-
13.	Terpenoids	Red brown colour	-

Phytochemical analysis of Centella asiatica in solvent Ethyl acetate

SL.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	Alkaloid test	Orange red colour formation	+
2.	Coumarins test	Yellow colour formation	+
3.	Steroid test	Red colour formation	**-**
4.	Saponins test	Copious lather formation	**-**
5.	Flavones test	Red colour formation	**-**
6.	Tannins test	Dark blue or greenish black	+
7.	Quinones test	Red colour formation	-
8.	Flavanones	Yellow to orange Orange to crimson red	-
9.	Anthocyanins	Blue colour yellowish orange	-
10.	Phenols	Blue or green colour	-
11.	Proteins	Violet formation	-
12.	Glycosides	Brown ring at the interface	-
13.	Terpenoids	Red brown colour	-

Phytochemical analysis of *Centella asiatica* in solvent Methanol

SL.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	Alkaloid test	Orange red colour formation	+
2.	Coumarins test	Yellow colour formation	+
3.	Steroid test	Red colour formation	**-**
4.	Saponins test	Copious lather formation	**-**
5.	Flavones test	Red colour formation	+
6.	Tannins test	Dark blue or greenish black	+
7.	Quinones test	Red colour formation	**-**
8.	Flavanones	Yellow to orange Orange to crimsonred	-
9.	Anthocyanins	Blue colour Yellowish orange	-
10.	Phenols	Blue or green colour	+
11.	Protiens	Violet formation	-
12.	Glycosides	Brown ring at the interface	-
13.	Terpenoids	Red brown colour	-

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