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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS OF *CENTELLA ASIATICA* L. LEAF EXTRACTS.

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Abstract

Centella asiatica (L) commonly known as Indian Pennywort is having great medicinal value and hence used as a medicinal herb in Ayurvedic medicine. The present study intends to provide an overview of the chemical constituents present in the crude leaf extracts of *C. asiatica* (L) with special emphasis on their pharmacological actions. Phytochemical screening of *C. asiatica* (L) was studied by extracting the dried powdered leaves with four different solvents such as water, acetone, chloroform and methanol. The extracts were subjected to qualitative phytochemical analysis using standard procedures. Preliminary phytochemical analysis revealed the presence of eight compounds such as carbohydrates, tannins, steroids, terpenoids, alkaloids, flavanoids, cardiac glycosides, saponins etc. Phytochemical analysis of the dried samples were more positive for methanol extracts. The results suggest that the leaves of *C. asiatica* (L) are a rich source of valuable primary and secondary metabolites.

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Introduction:-

Centella asiatica (L) Urban syn. synonym *Hydrocotyle asiatica* (L) commonly known as Indian Pennywort belongs to the family Apiaceae (previously known as Umbelliferae)¹. 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, flavonoids, saponins, reducing compounds, minerals and vitamins².

The plant is a perennial herb with creeping stem, rooting at the nodes and with simple, reniform, long arachnoid petioled leaves. Inflorescence is glabrate to finely arachnoid. Involucre of 2 ovate bracts. Umbels 2-4 flowered. Flowers pink. Calyx teeth obsolete. Stylopodium depressed, purplish; styles short. Fruit ovate to orbicular, 2-3 mm long; primary ridges prominent, secondary forming a network; vittae not distinct^{3,4,5}.

C. asiatica (L) is one of the important medicinal plants in the International market of medicinal Plant Trade. 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, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Medicinal herbs have been in use in one form or another, under indigenous system of medicine like

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Ayurveda, Siddha and Unani. The plant is known as 'thankuni' in Bengali, 'Indian pennywort' in English, 'gotu kola' in Sinhala, 'mandukaparni' in Sanskrit (Ayurveda), 'Hydrocotyle asiaticus' in French, and 'Asiatischer Wassernabel' in German⁶.

Phytochemicals are plant derived chemicals, which may protect human from a host of numerous diseases. These chemicals are naturally occurring in medicinal plant leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds⁷. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities⁸. Terpenoids are very important in attracting useful mites which would consume the herbivorous insects⁹. Alkaloids are used as anaesthetic agents and are found in medicinal plants¹⁰. The present study is aimed to assess the phytochemical constituents in methanol, acetone, chloroform and aqueous extracts of the leaf extracts of *C. asiatica* L.

Materials and Methods:-

Collection of plant Material:-

Fresh plant/plant parts of *C. asiatica* L were collected randomly from pathanamthitta, Kerala, India. The taxonomic identities of the plant was confirmed by Dr. Neethu S Kumar at Post Graduate Department and Research centre of Botany, Mahatma Gandhi College, Kesavadasapuram, Kerala University. Fresh fully matured leaves were washed thoroughly under running tap water, shade dried, homogenized to fine powder and stored in air tight bottles for future use.

Preparation of plant extracts:-

The dried powdered leaves were extracted with four different solvents such as water, acetone, methanol and chloroform. For aqueous extraction, five grams of the powdered leaves were mixed with 50ml of distilled water, boiled for about two hours and filtered. Whereas acetone, methanol and chloroform extracts were prepared by mixing five grams of powdered leaf samples with 50ml of each solvent separately in an orbital shaker incubator for about 48 hours at room temperature. Extracts were filtered, concentrated, dried and stored in the refrigerator at 4°C for further use.

Phytochemical Analysis:-

The major secondary metabolites like carbohydrates, tannins, steroids, terpenoids, alkaloids, flavanoids, glycosides, saponins, quinons and phenolic compounds were assessed¹¹. The presence of phytochemicals extracted in various solvents was confirmed by standard protocols.

Test for Alkaloids:-

Wagner's reagent test

A fraction of the extract was treated with 3 -5 drops of Wagner's reagent and observed for the formation of reddish brown precipitate (or colouration) which indicates the presence of alkaloids.

Test for Carbohydrate:-

Molish's test:-

To 2 ml of Molish reagent, 2 ml of the extract were added and shaken well. To this 2 ml. of conc. H₂SO₄ was added through the sides of the test tube. Appearance of a reddish violet ring at the junction of the two layers indicate the presence of carbohydrates.

Fehling's Test:-

The filtrate was treated with 1 ml. of fehling's A and B and heated in a boiling water bath for 5 -10 minutes. Appearance of a reddish orange precipitate shows the presence of carbohydrates.

Detection of Flavanoids:-

The extracts were treated with conc. H₂SO₄ and observed for a yellowish orange colour for the presence.

Test for cardiac Glycosides:-

Added 1ml. of extract with 1 ml. of glacial acetic acid and 2-3 drops of 5% ferric chloride solution. To this mixture were added 0.5 ml. dilute HCl. Appearance of a green ring which first turns to violet and then to brown at the interface indicates the presence of cardiac glycosides.

Test for protein:-**Biuret Test:-**

One ml of 40% NaCl. and 2 drops of 1% CuSO_4 were added to the leaf extracts. Appearance of a violet colour confirms the presence of proteins.

Test for phenolic Compounds:-**Ferric chloride Test:-**

Two ml of diluted extracts were treated with dil. FeCl_3 solution. Appearance of a violet colour indicate the presence of phenol like compounds.

Test for quinones:-

A small amount of the extract was treated with conc. HCl. and observed for the formation of a yellow precipitate colouration.

Test for Saponines:-

Two ml of the extracts were diluted with 20 ml of distilled Water, shaken vigourously and was observed for a stable persistent froth.

Test for steroids:-

To 1ml. of solvent extract in a test tube acetic anhydride was added and kept in a boiling water bath for 5min, then cooled followed by the addition of 1ml of Con. H_2SO_4 along the sides of the test tube. Appearance of a green color indicate that the occurrence of steroids

Test for tannins:-

To the extracts were added a few drops of 10% ferric chloride solution. Appearance of a green/yellow colour indicates the presence of tannins.

Test for terpenoids:-**Salkowski's Test:-**

Two ml of the extracts were mixed with 1 ml of chloroform and of conc. H_2SO_4 solution. A reddish brown colour at the inter phase indicates the presence of terpenoids.

Result and Discussion:-

Result obtained for qualitative screening of phytochemicals in the leaves of *C. asiatica* (L) and its bioactivity are presented in table 1 & 2. The extracts of *C. asiatica* (L). showed different phyto profiles with reference to the solvents. Out of the four solvents (methanol, acetone, chloroform, water) used methanolic extract demonstrated the maximum occurrence of phyto constituents (8/11) such as alkaloids, flavanoids, glycosides, phenolic compound, steroids, terpenoids etc.

Table 1:- Phytochemical screening of *C. asiatica* (L).

Test	Solvents			
	Methanol	Acetone	Chloroform	Water
Alkaloids	+	+	+	+
Carbohydrate	-	-	-	+
Flavanoids	+	-	+	-
Glycosides	+	+	+	+
Phenolic compound	+	-	-	+
Protein	-	-	-	-
Quinones	-	-	-	-
Saponins	+	-	-	-

Steroids	+	-	-	-
Tannins	+	+	-	-
Terpenoids	+	+	-	+

Phytochemical screening of the extracts of *C. asiatica L.* revealed the presence of alkaloids, flavanoids, glycosides, phenol, saponins, steroids, tannins and terpenoids. These compounds have significant application against human pathogens.

Table 2:- Activity of phytochemicals ^{12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25}

Phytochemicals	Activity
Quinones	Antimicrobial
Flavonoids	Antimicrobial Antidiarrhoeal
Polyphenols and Tannins	Antimicrobial Antidiarrhoeal
Terpenoids	Antimicrobial Antidiarrhoeal
Alkaloids	Antimicrobial Antidiarrhoeal
Glycosides	Antidiarrhoeal
Saponins	Antidiarrhoeal Anticancer
Steroids	Antidiarrhoeal

Conclusion:-

The present study clearly indicates that the methanol crude extracts of *C asiatica (L.)* exhibited the highest zone of inhibition than compared to the other extracts like water, chloroform and acetone. Many evidences gathered in earlier studies had already confirmed the identified phytochemicals to be bioactive. Several studies in turn confirmed their contribution to the medicinal as well as physiological properties of the plant towards the treatment of different ailments. Medicinal plants thus play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Phytochemical analysis of the medicinal plants are hence commercially significant in both research institutes as well as pharmaceutical companies for the manufacturing of new drugs for the treatment of various illness. Studies conducted by previous workers and the present study show nearly similar results related to the phytochemical constituents in the leaf extracts of *C. asiatica (L.)*. It would not be surprising therefore to use plant samples to cure certain types of illness in humans and animals. This obtained information will therefore serve as a primary platform for further phytochemical and pharmacological studies related to the concerned plant. Hence it can be concluded that the leaves of this herb would direct to the establishment of some compounds that could be used to invent new and more potent anti microbial drugs of natural origin. Therefore future research should be addressed on the application of using leaves of the aforesaid medicinal herb as natural remedied and to protect against infectious diseases.

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