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Research Article

PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF DIFFERENT SOLVENT EXTRACTS OF *CENTELLA ASIATICA* L. (FAMILY: APIACEAE), AN ETHIOPIAN WEED

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ABSTRACT

The conventional medicine system engages the exercise of various plant extracts or active principles. Ethno medical studyintenselysignifiesthat one of the greatestopportunityin searching novel cost-effective plants for medication. This investigation generally provides the healthclaim at reasonable price. The present study is aimed to assess the preliminary qualitative and quantitative phytochemical constituents in methanol, hexane and aqueous extracts of *Centella asiatica*L. The qualitative preliminary phytochemical screening of methanol, hexane and aqueous extracts showed the presence of alkaloids, saponins, Total phenols and tannins, steroids and absence of remaining secondary metabolites. The total alkaloidsestablishedin the aqueous extract was 2.25mg/g and total saponin content was FI < 100. The phytochemicals recognized in this present investigationcould be utilized as tools for drug quality control obtained from*C.asiatica*in the future, for the healing of a diversity of disease circumstances.

Keywords: Centella asiatica**,** phytochemical, traditional medicine.

INTRODUCTION

Phytochemicals are plant derived chemicals, which may protect human from a host ofnumerous diseases. They are non-nutritive plant derivatives that are capable to prevent various dreaded diseases[1].In general, the plant manufactures theseactive chemicals to protect itself.However thecurrent researchsuggests that these phytochemicals candefend human diseases. Phytochemicals are secondary metabolites obtain from the leaves, stem, roots, fruits and whole herbs, which synthesizedifferent quantity of bioactive compounds based on the habitat and adaptation[2].These medicinal compounds are safe and eco-friendly. According to WHO, about75% of the world's inhabitants relies on conventional medicine for their chief health care[3]. In the customary system of medicine, most of theplant extracts are helped to cure a diversity of the non-communical and communicable diseases[4].

Centella asiatica L. (Family Apiaceae) is a significant medicinal herb employedbased on the familiarity, which is very popular in most tropical and subtropical countries[5]. It is clonal, perennial creeper found in all over Africa, growing in swapy places. It is commonly known

as Asiatic pennywort containing 20 species grows fastly in most parts wetrocky and higher elevations[5]. The whole plant parts are used as medicinal values. The leaves areextensively utilized as a blood purifier, memory enhancementand for treating elevated blood pressure and prevent ageing[6]. In Ayurvedic system of medicine, the whole plant is one of the main herbal constituents for invigorating the nerves and brain cells. Further, the plant used to treat depression, wound inflammation, gastric ulcer, epilepsy, leprosy, cognitive disorders and venomous bites[7-11].The present study is aimed to assess the phytochemical constituents in methanol, hexane and aqueous extracts of *Centella asiatica* L. and qualtity assessment of active constituents such as alkaloids and saponins in leaves and stem of the plant.

MATERIALS AND METHODS

CHEMICALS

Ferric chloride, HCl, Dragendorff 's reagent, hexane, benzene, carbon tetrachloride, chloroform, H_2SO_4, Folin-Ciocalteu reagent were purchased from Chemico Glass & Scientific Company, Erode, Tamilnadu, India. All the chemicals used in this experiment were of analytical grade.

Collection and authentication of plant material

*Centella asiatica*L. was collected from Jimma University Garden, Jimma, South West Ethiopia in the month of October-2014. The plant has been taxonomically identified and authenticated by the Jimma University Botanist Dr. Ramesh and kept in Jimma University Botanical Science and Herbarium for future references.

Methanol extract of leavesand stem of *Centella asiatica*

The shadow dehydrated roughly powdered of leaves and stem of *C. asiatica*was engrossed and haul out with methanol for 72hrs. After finishing point, the defatted solutions were sieved by filter paper Whatmann No.1 to eliminate any contamination. The extract was intensed by vaccum dessicator to reduce the degree; the concentrated samples were relocated to another beaker and the residual solvent was further vaporized. Finally the dark greenish yellow coloured extract was formed and again it was kept in a vaccum dessicator to get rid of unnecessary wetness. Dehydrated extract was stored in sealed a container and which was used for qualitative phytochemical screening.

Aqueousand hexane extracts of leavesand stem of *C. asiatica*

The resudues left after methanol extraction was dehydrated and then engrossed separately with aqueousand hexane respectively up to 3days. After finishing point of extraction, the organic solvents were eliminated by vaccum dessicator. Dark greenish yellow colour extracts were formed and then stored in a sealed container for further studies.

Organoleptic evaluation

Organoleptic characters of the plant samples were determined by the technique depicted by Siddiqui and Hakim[12]. Organoleptic evaluation demostrated to evaluate methanol, aqueous and hexane extracts of the leaves and stem of *Centella asiatica*L. by its odour, colour, taste and texture.

Preliminary phytochemical studies[13, 14]

The extracts obtained (benzene, ethanol, carbon tetrachloride, and aqueous) were employed to the subsequent phytochemical screening.

Test for Alkaloids

a) **Dragendorff's test**
Take 1ml of the solvent extract, add equal volume of distilled water followed by 1ml of 2molar solution of HCl added until acidification reaction takeplace. To Add this 1ml of Dragendorff's reagent. Orange or red colour is formed, indicated that the occurrence of alkaloids.

b) **Hagger's Test**
Take 1ml of the solvent extract in a cleaned test tube, add 1ml of Hager's reagent. Yellow precipitate is formed, indicated that the occurrence of alkaloids.

c) **Wagners Test**
Take1ml of solvent extract acidified with 1ml of 1.5 % v/v of HCl and add 1ml of wagners reagent. Formation of yellow or brown precipitate, which indicated that the occurrence of alkaloids.

d) **Mayers Test**
Take 1ml of Mayers reagent, add 1ml of solvent extract. White or pale yellow precipitate is formed, indicated that the occurrence of alkaloids.

Test for Carbohydrates

a) **Anthrone Test**
Take 1ml of solvent extract and 10ml of distilled water in a test tube, shaken vigorously and filtered. To this filtrate,

add 1ml of anthrone reagent and mixed. Green or blue color is formed, indicated that the occurrence of carbohydrates.

b) Benedicts Test
Take1ml of solvent extract and 10ml of water in a test tube, shaken vigourously and filtered. To this filtrate, add 3ml of Benedicts reagent and kept in a boiling water bath for 5min. Development of red colour indicated that the occurrence of reducing sugar.

c) Fehlings Test
Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigourously and filtered. To this filtrate, add 1ml of Fehlings solution A and 1ml of Fehlings solution B and kept in a boiling water bath for 5min. Development of red colour indicated that the occurrence of reducing sugar.

d) Molischs Test
Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigourously and filtered. To this filtrate, 1ml of Molisch reagent were added followed by few drops of Conc. H_2SO_4 added in the side of the test tube. Formation of two junction, which indicates the occurrence of carbohydrates.

Test for flavonoids

a) Shinods test
Take 1ml of solvent extract diluted with 3ml of ethanol followed by 2ml dilute HCl and pinch of Mg in a test tube, shaken gently. Appearance of pink or brown precipitate indicated that the occurrence of flavonoids.

b) With Con. H_2SO_4 test
when treated with Con. H_2SO_4, appearance of the following colour like yellow colour (anthocyanins), yellow colour change to orange (flavones); orange colour change to crimson (flavonones) respectively.

Test for Glycosides

Molisch Test
Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigourously and filtered. To this filtrate, 1ml of Molisch reagent were added followed by few drops of Conc. H_2SO_4 added in the side of the test tube.. Formation of two junction, which indicates the occurrence of glycosides.

Test for proteins and free amino acids

1. **Millions test**
Take 1ml of solvent extract with 1ml of Millions reagent, shake gently. Appearance of cherry red color indicated that the occurrence of free amino acid.
2. **Ninhydrin test**
Take 1ml of solvent extract with 1ml of Ninhydrin reagent, shake gently Formation of violet color indicated that the occurrence of free amino acids.
3. **Biuret test**
Take 1ml of solvent extract with 1ml of 10% NaOH and 1ml of 1% copper sulphate in a test tube, shake gently. Development of purple color indicated that the occurrence of proteins.

Test for gums and mucilage

With 95% alcohol
Take 1ml of solvent extract with 25 ml of 95% alcohol in a test tube, shake gently and filtered. The residue was air dried and examined for its bulging property. It indicated that the occurrence of gums and mucilages.

Test for anthraquinones
Take 2ml of the solvent extracts acid hydrolysed with Conc. H_2SO_4 followed by extracted with benzene. Add 2ml of dilute ammonia. Appearance of rose pink color indicated that the occurrence of anthraquinones.

Test for Saponins
Foam test : Take 5ml of solvent extracts in a test tubes add a drop of sodium bicarbonate, shaken vigorously and kept it stand for 3min. Development of cloudy white precipitate indicated that the occurrence of saponins.

Test for Sterols

a) Liebermann-Buchards test
Take1ml of solvent extract in a test tube and add acetic anhydride and kept in a boiling water bath for 5min, then cooled followed by 1ml of Con. H_2SO_4 added along the sides of the test tube. Appearance of green color indicated that the occurrence of steroids.

b) **Salkowski reaction**
Add 1ml of solvent extract diluted with chloroform and followed by 1ml of Con. H_2SO_4 added along the sides of the test tube. Appearance of two junction/layer indicated that the occurrence of steroids.

Test for fixed oils

Spot test

Take 0.5ml of solvent extract and pressed in between the two filter papers. Formation of oil stains on the paper indicated the existence of fixed oil.

Add 1ml of 0.5N alcoholic KOH and 1ml of solvent extract along with a single drop of phenolphthalein in a test tube. The residues were kept in a boiling water bath for 20min. Appearance of soap or incomplete neutralization of alkali indicated that the occurrence of fixed oils.

Test for triterpenoids

Add 2ml of solvent extract and 1 ml of $CHCl_3$ followed by 1 ml of acetic anhydride in a test tube and shake gently. Add 1ml of Con. H_2SO_4 added along the sides of the test tube. Appearance of two junction/layer indicated that the occurrence of triterpenoids.

Test for phenolic compounds and tannins

About 5ml of solvent extracts and equal volume of water added and perform the following reagent for confirmation of phenolic compounds and tannins.

Ferric chloride reagents

It givesa violet color.

Gelatin containing sodium chloride

It givesa white precipitate.

Lead acetate solution

It givesawhite precipitate

Quantitative estimations

Estimation of Total Alkaloid

Quantitatively, alkaloid was determined using the procedure forward by Harborne, 1973; as described by Edeoga et al.[15]. Briefly, 5 grams of dried leaves and stem ofthe plant powder was kept into 200 ml of 20% acetic acid was added and kept to stand for 4hrs at room tempearature. Then the extract were filtered and the extract was concentrated by using a water bath to one quarter of the volume. In that, Con. ammonium hydroxide was added drop by drop until the precipitate formed. Then the precipitated solution was allowed to filetreed by whatmann filter paper No.1 and residue on the filter paper dried at oven and weighed accurately. Total alkaloid content was calculated as mg per g of air-dried material.

Estimation of Total saponin (Determination of foaming index)

Most of the medicinal plants contain saponins, that can produce persistent foam when present in an aqueous medium with shaking. The foaming ability of an aqueous medium of plants and their extracts wascalculated in terms of a foaming index. Saponins were determined according to the method described by World Health Organization[16]. Reduce about 1gram of the plant powder weighed accurately and mixed with 100 ml of boiling water. Continue at moderate boiling temperature for 30 minutes.Then cooled and filtered and added sufficient water through the filter to dilute to volume. Added the decoction into 10 test-tubes in series of successive portions of 1,2, 3... 10 ml and the volumes in each tube adjusted with water to 10ml. The tubes were shaken them in a lengthwise motion for 15 seconds. After allowed the tubes to stand for 15 minutes and the height of the foam was measured by means of a graduated tape with millimetre scale.

Statistical analysis

Quantitative estimation of the secondary metabolites carried out by statistical analysis which is conceded by Microsoft Excel 2007 software and average values along with SD were calculated.

RESULTS AND DISCUSSION

The Organoleptic characters of aqueous, methanol and hexane extracts of the *Centella asiatica* L.were shown in Table.1. The aqueous, methanol and hexane extracts of leaves and stem of *Centella asiatica* L.had similar organoleptic properties (Table-1). The qualitative phytochemical screening of aqueous, methanol and hexane extracts of stem and leaves of *Centella asiatica*L.and its secondary metabolites were shown in Table-2. The results showed the presence of phytochemical constituents, namely alkaloids, saponins, steroids, total phenols and tannins, triterpenoid, and absence of proteins and aminoacids, flavonoids, glycosides, anthoquinones, and steroids (Table 2). The quantitative test for active constituents such as alkolids and saponins of stem and leaves of *Centella asiatica*L.were shown in Table-3. The total alkaloid contents were found to be 2.25 mg/gand total saponin content was (Foaming Index) FI < 100 (Table-3).

The initial phytochemical screening tests may be helpful in the screening of the bioactive compounds and eventually may help to detection and development of new drugs. Further, these tests make easy their qualitative

separation and quantitative estimation of pharmacologically active chemical compounds.[17] The phytochemical screening in the present study has publicized the presence of alkaloids, saponins, total phenols and tannins, and triterpenoids in the leaves, and stem extracts. Further the presence of different phytochemicals in the aqueous and two different organic solvent extracts may be responsible for the therapeutic properties of *C. asiatica*.

Tannins is a phenolic compounds that are acting as principal antioxidants or free radical scavengers. Since these phenolic compounds were originated to be present in the extracts, it might be accountable for the potent antioxidant capacity of *C. asiatica*. These phytochemicals of medicinal plants have primarily reported for their medicinal value, which can be valuable for therapeutic index. For instance, saponins proved as hypotensive and cardiodepressant properties,[18] which are helpful for the treatment of congestive heart failure and cardiac myopathy.[19] The occurrence of saponins in methanol and aqueous extracts of leaves and stem of *C. asiatica*might play a role in the cardioprotective potential. Alkaloids and tannins have the potential of hypoglycemic and anti- inflammatory activities.[20]Moreover, the terpenoids have also been revealed to decrease blood sugar level in animal studies.[21] In addition, the steroids and triterpenoids demonstrated the analgesic properties and central nervous system activities.[2, 22, 23]Hence the preliminary phytochemical investigation are actually obliging in finding chemical ingredients in the plant that may help to their quantitative evaluation and also in locating the source of pharmacologically active principle.

CONCLUSION

Since the study was conducted in a controlledmanner, the phytochemical results can beused for the standardization of the abovementioned drugs. A preliminary screening andmore research has to be undertaken to explorethe wonderful therapeutic properties of thesemedicines. To conclude the presence study,we have found that most of the biologicallyactive phytochemicals were present in methanol, hexane and aqueous extracts of leaves and stem of *C. asiatica*.

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Table 1: Organoleptic properties of aqueous, methanol and hexane extracts of whole plant of *Centellaasiatica* L

Plant parts	Name of the extracts	Appearance	Colour	Taste	Odour
Leaves	Aqueous	Liquid	Emerald green	Tasteless	Characteristic
	Methanol	Liquid	Green	Tasteless	Characteristic
	Hexane	Liquid	Green	Tasteless	Characteristic
Stem	Aqueous	Liquid	Emerald green	Tasteless	Characteristic
	Methanol	Liquid	Green	Tasteless	Characteristic
	Hexane	Liquid	Green	Tasteless	Characteristic

Table 2: Total alkaloids and saponin contents in powder of *Centellaasiatica* L.

Name of the plant material	Total alkaloids	Total saponins (Foaming Index)
Leaves and stem powder of *Centellaasiatica* L.	2.25±0.01	FI < 100

Table 3: Phytochemical investigation of leaves and stem of *Centellaasiatica* L. using aqueous, methanol and hexane solvents

Analysis	*Centellaasiatica*L.					
	Leaves			Stem		
	Methanol	Hexane	Aqueous	Methanol	Hexane	Aqueous
Alkaloids	--	+++	+++	-	+++	-
Protein and aminoacids	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-
Saponins	++	-	+++	-	-	++
Steroids	+	-	+	-	-	-
Total phenols and Tannins	++	-	+++	+	-	++
Triterpenoids	-	-	-	-	-	-

+++ = appreciable amount (positive within 5 mins.); ++ = moderate amount (positive after 5 mins. but within 10 mins);
+ = trace amount (positive after 10 mins. but within 15 mins); - = completely absent.

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