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Pharmacognostic review and phytochemical screening of *Centella asiatica* Linn.

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Abstract

Various studies have already been performed involving the whole aerial parts of *Centella asiatica* (L.) (Umbelliferae), commonly known as gotukola or jalbrahmi and thus the present investigation has been carried out for the phytochemical study of ethanolic extract of the aerial parts of *Centella asiatica*. To perform this activity, the drug (1.5kg) was exhaustively extracted in 95% ethanol using Soxhlet apparatus. The Paper deals with the study of its phytoconstituents, pharmacological activities as well as the phytochemical screening of the plant.

Keywords: Whole aerial parts, *Centella asiatica*, Soxhlet, asiaticosides, nervine activity

1. Introduction

Centella asiatica (Hydrocotyl asiatica), belonging to family Umbelliferae, also known as gotukola in Hindi ^[1]. It is known as karinga in Marathi and mandukparni in Sanskrit ^[2]. It is a prostrate, slender, tender, faintly aromatic herb, which has numerous creeping stoloniferous stems, rooted at nodes with long internodes.³ The plant contains the glycosides viz Asiaticosides A & B, madecassosides and centellosides.⁴ It contains the triterpene acids such as Asiatic and madecassic acid ^[5]. Flavanoids such as kaempferol and quercetin are also present in the plant ^[6]. The plant contains volatile and fatty oil. The fatty oil consists of glycerides of palmitic, stearic, lignoceric, oleic acids ^[7]. Centella is also rich in Vitamin C, Vitamin B1, Vitamin B2, niacin, carotene and Vitamin A. The total ash contains chloride, sulphate, phosphate, iron, calcium, magnesium, sodium, potassium etc ^[8, 9]. The plant shows various pharmacological activities such as gastric ulcer healing activity, which is shown by asiaticoside present in it ^[10]. Its crude methanolic extract shows antioxidant activity ^[11]. In Indian medicine the plant is important as a tonic for skin diseases and leprosy, and is reported to promote fibroblast proliferation and collagen synthesis ^[12]. The plant also serves for its psychotropic uses ^[13]. Alcoholic extract of the plant shows anti-protozoal activity against *Entamoeba histolytica* ^[14]. Crude water extracts of Centella plant along with other plant combinations show anti-herpes simplex virus activities ^[15]. Majority of studies have been performed on the various parts of *Centella asiatica*, so the present study involves the phytochemical investigation of ethanolic extract of the whole aerial parts of *Centella asiatica*.

2. Reported Phytoconstituents

Centella asiatica is reported to contain following type of compounds:

1. **Glycosides:** Asiaticoside A & B, madecassoside & centelloside have been isolated from the plant. On hydrolysis, these glycosides yield the triterpene acids-asiatic acid, centellic acid. The variation in the chemical composition of Indian samples of the plant is attributed to geographical conditions ^[4].
2. **Triterpene acids:** The plant contains the following triterpene acids: asiatic, madecassic, terminalic, centic, centellic, centoic acid, betulic, brahmie & isobrahmic acid ^[5].
3. **Flanonoids** 3- β glucosyl quercetin, 3- β glucosylkaempferol, 7- β - glucosylkaempferol, kaempferol & quercetin have been isolated from the leaves ^[6].
4. **Alkaloids:** An alkaloid hydrocotylin has been isolated from the dried plant.
5. **Volatile & Fatty oil:** The plant contains volatile & fatty oil. The fatty oil consists of glycerides of palmitic, stearic, lignoceric, oleic, linoleic & linolenic acids ^[7].

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Others The plant is reported to contain polyacetylenes, carotenoids, vitamin B, vitamin C, tannins, sugars, inorganic acids, resins. The plant also contains amino acids viz. aspartic acid, glycine, γ -alanine, phenylalanine. The total ash contains chloride, sulphate, phosphate, iron [3].

Chemical structures of Reported Compounds of *Centella asiatica* (Linn.)

Glycosides

Asiaticoside A X = glucose (6-1) glucose (4-1) rhamnose

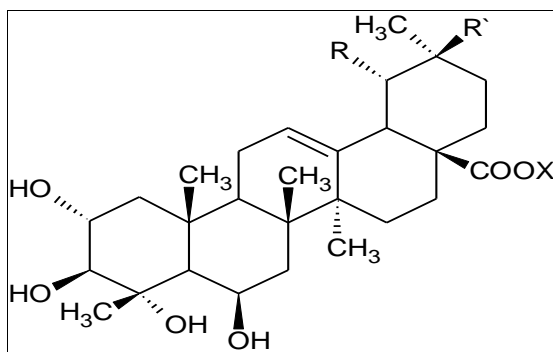


Fig 1: R=methyl

Asiaticoside B X= glucose (6-1) glucose (4-1) rhamnose
R= H, R' = methyl, Madecassoside.

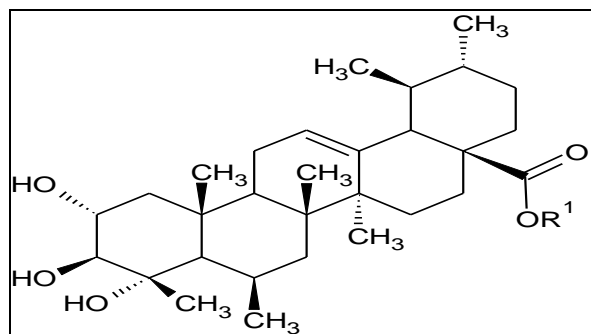


Fig 2: R= OH

Centelloside
Brahminoside

B) Triterpene Acids

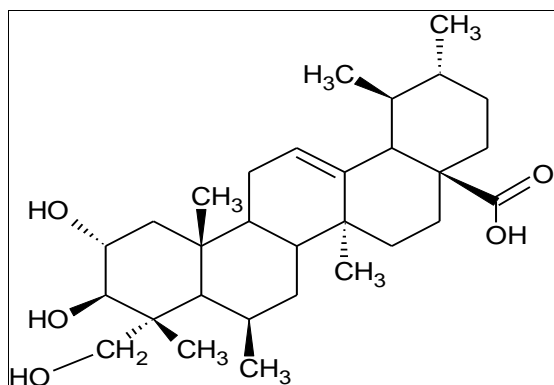


Fig 3: Brahmic acid

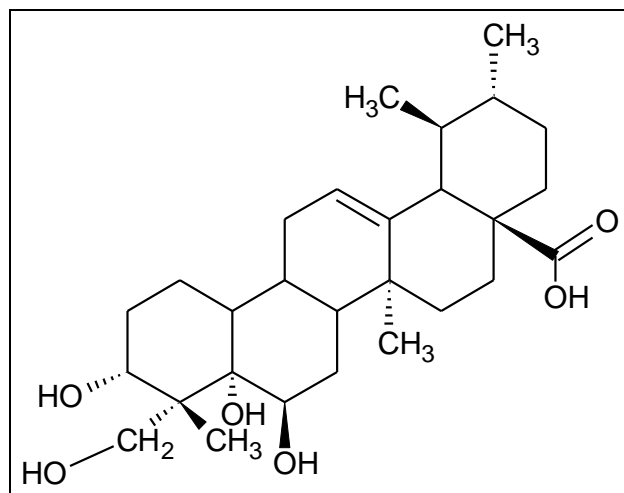


Fig 4: Isothankunic acid

- Betulic acid
- Centic acid
- Centoic acid
- Isobrahmic acid

C) Flavones

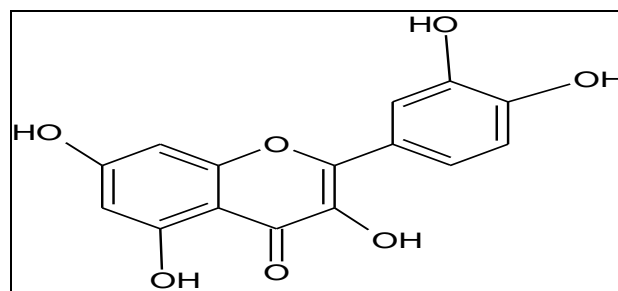


Fig 5: Kaempferol

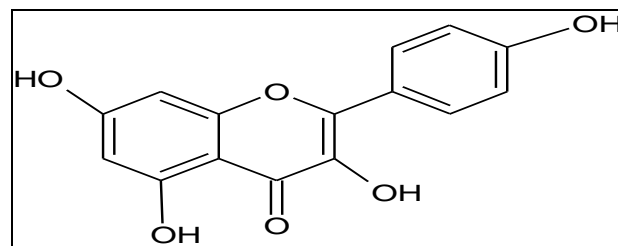


Fig 6: Quercetin

D) Alkaloids

- Hydrocotylin ($C_{22}H_{33}NO_8$)

E) Volatile and fatty oil

Glycerides of –

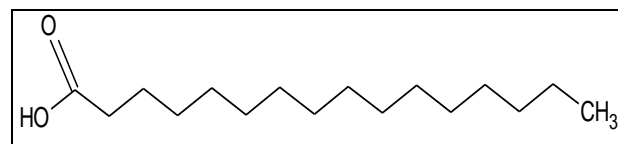


Fig 7: Palmitic acid

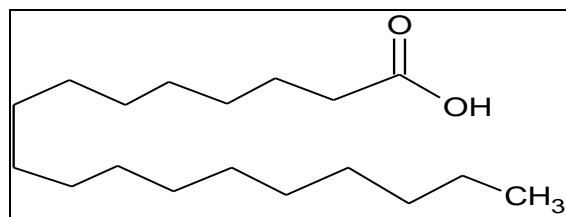


Fig 8: Stearic acid

- Lignoceric acid
- Oleic acid
- Linoleic acid
- Linolenic acid⁷

3. Pharmacological Activities

Dermal wound healing activity is shown by the alcoholic extract of the plant consisting madeassic acid, asiatic acid & asiaticoside^[6].

Gastric ulcer healing activity - is shown by asiaticoside, which prevents development of cold induced gastric ulcer in rats. Aqueous extract shows healing effects on acetic acid induced gastric ulcer in rats^[10].

Memory enhancing activity is shown by the aqueous extract of the herb. It showed significant effect on learning & memory and decreased the levels of non-epinephrine, dopamine & 5-HT in the brain.

Antioxidant activity is shown by the crude methanolic extract on lymphoma bearing mice^[11].

Immunomodulating activity is shown by the triterpenoidal saponins of the plant. The WBC count is significantly increased.

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Antidepressant activity is shown by the total terpenes in forced swimming mice & ameliorated the balance of amino acid levels. They increased the contents of monoamine neurotransmitters in rat brain.

Anti-fertility activity in female mice is shown by the crude extract of the plant & glycosides isothankuniside.

Anti-Inflammatory activity -is shown by the aqueous extract of the plant due to asiaticoside by the inhibition of NO synthesis & thus facilitate ulcer healing^[3].

Antispasmodic activity is shown by the alcoholic extract of the plant on acetylcholine induced contractions of rat ileum, due to its glycosides

Anti-Tubercular & antileprotic activity - is shown by the active constituent asiaticoside of the plant^[7].

4. Medicinal uses The plant is cooling, alterative, cardiogenic, nervine tonic, sedative to nerves, stomachic, carminative, improves appetite, antileprotic, memory enhancer. It is given for the treatment of mental illness, insomnia, and epilepsy.

The leaves are useful to treat ulcer, eczema, psoriasis, leprosy, TB, cardiac debility, asthma & fever.

The leaves are also useful in abdominal disorders due to dysentery in children.

In Chinese medicine the herb is used for dysentery, summer diarrhoea, vomiting, jaundice, scabies etc.

In Homeopathic medicine it is used for skin diseases associated with itching and swelling. It is used to treat inflammation, ulceration of uterus, eczema, elephantiasis etc.^[3].

5 Preliminary Phytochemical Screening

The ethanolic extract was subjected to preliminary phytochemical investigation for the detection of the following metabolites:

- Alkaloids
- Carbohydrates
- Glycosides
- Phenolic compounds
- Flavonoids
- Protein and free amino acids
- Saponins
- Sterols

Tests for alkaloids

Five milliliter of the ethanolic extract was evaporated to dryness. The alcoholic residue were taken in 5 ml of 2% hydrochloric acid, saturated with sodium chloride

1. Mayer's reagent (KI + Hg₂Cl₂ solutions), with it creamish white precipitate is obtained-shows presence of alkaloids.
2. Dragendorff's reagent (excess of KI + BiNO₃ solution) with it - red brown precipitate - alkaloids present.
3. Wagner's reagent - Red brown precipitate confirms the presence of alkaloids.
4. Hager's reagent - No yellow colour is produced.

Test for Carbohydrates

- (a) **Molisch Test:** To 2 ml of alcoholic extract few drops of α -naphthol (20% in ethyl alcohol) was added. Then 1 ml of conc. sulphuric acid was added along the side of the test tube. Reddish violet ring at the junction of the two layers shows carbohydrate presence.
- (b) **Reduction of Fehling's Solution:** 1 ml of fehling's solution (copper sulphate in alkaline conditions) was added to the concentrated ethanolic acid & heated on a steam bath. No brick red precipitate is produced, i.e no reducing sugar is present.

Test for glycosides - 2 millilitre of alcoholic extract was taken & subjected to following tests_

- a. **Keller-Killiani Test-** One milliliter of glacial acetic acid containing traces of ferric chloride & 1 millilitre of conc. sulphuric acid were added to the extract carefully. Reddish brown colour formation at the junction of two layers & the upper layer turn blue green. This confirms the presence of glycosides.
- b. **Bornstrager's Test** One milliliter of benzene & 0.5 millilitre of dilute ammonia solution were added to the ethanolic extract. No reddish pink colour is produced.
- c. **Legal Test** Concentrated ethanolic extracts was made alkaline with few drops of 10% sodium hydroxide and then freshly prepared sodium nitroprusside solution was added to the solution. Blue color is produced which shows the presence of carbohydrates.

Test for Phenolic Compounds

3 ml of ethanolic extract was evaporated to dryness, extracted with 5ml of distilled water, 5% ferric chloride solution was added. Blue green colour was produced which shows the presence of phenolic compounds.

(a) Lead Acetate test

3 drops of lead acetate solution (5%) were added to the aqueous

extract yellow precipitate is produced, which shows the presence of phenolic compounds.

(b) Gelatin Test

2 ml of 1% of gelatin solution was added to aqueous extract. Precipitate/Turbidity is produced which confirms the presence of phenolic compounds.

Test for Flavonoids

(a) Ammonia Test

Filter paper strips were dipped in the alcoholic solution of the extract and ammoniated. Strips turn yellow and it shows the presence of flavonoids.

(b) Shinoda/Pew Test

In 1ml extract a piece of metallic zinc was added, followed by addition of 2 drops of hydrochloric acid. Deep red colour is produced which confirms the presence of flavonoids.

Test for Proteins and Free Amino Acids

Xantho Protein Test

To 2ml of extract, 3 drops of nitric acid were added by the side of the tube, yellow color is produced, which confirms the presence of Proteins and Amino acids.

Test for Saponins

To the alcoholic extracts 3 drops of sodium bicarbonate were added and shaken well. Honeycomb like frothing confirms the presence of saponins.

Test for Sterols

a. Salkowski Reaction

To the extract, 2ml of concentrated sulphuric acid was added. No yellow ring at the junction which turned red after 1 minute which shows the presence of sterols.

b. Hersche's Son's Reaction

2 ml of trichloroacetic acid was added to the extract. No red to violet color is produced which shows the absence of sterols.

Test for Acidic Compounds

- To alcoholic extracts, sodium bi-carbonate solution was added. No effervescence is produced which shows the absence of acidic compounds.
- Filtrate was tested with litmus paper and methyl orange. Blue colour doesn't changes to red confirms the absence of acidic compounds.

Table 1: The results of preliminary phytochemical screening.

S. No.	Phytoconstituents	Presence/Absence
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	+
4	Phenolic Compounds	+
5	Proteins and Free Amino Acids	+
6	Saponins	+
7	Sterols	-
8	Acidic Compounds	-

6. Results and Discussions

From the above literature review, it is concluded that *Centella* plant is an ample source of phytoconstituents such as Glycosides such as asiaticosides A and B, Triterpene acids such as brahmnic

acid and iso-brahmic acid, Flavones such as kaempferol and quercetin, Alkaloids such as Hydrocotylin, Volatile and fatty oil such as glycerides of palmitic, stearic acid etc. It shows a number of pharmacological activities such as anti-depressant, anti-inflammatory, memory enhancing, anti-oxidant etc. The results of the Phytochemical screening performed on the ethanolic extract of the plant shows the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, proteins but saponins, sterols and acidic compounds are absent.

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