



Pharmacognostic and Phytochemistry of *Centipeda minima*: A Review

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ABSTRACT: *Centipeda minima* is used as ethno-medicinal plant for treatment of various ailments. The present study was aimed to explore its pharmacognostic, fluorescence and Bio-chemical screening. The physical values like total ash, acid insoluble ash, water-soluble ash, alcohol soluble extractive and water-soluble extractives were determined. Air-dried powdered material has been subjected to qualitative and quantitative physicochemical estimations. The pharmacognostic evaluation total ash and quantitative analysis of leaves has revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats and carbohydrates. Its decoction is mostly used in paralysis and pain in the joints, and also against malaria, hepatitis, diabetes mellitus, eczema, insect or snake bites, and opium poisoning in lower shivalik hills of Himachal Pradesh. The natural stock of medicinal plants is under tremendous pressure. It must be conserved and promoted its commercial cultivation. Pharmacognostic and bio-chemical investigation also provides useful information in regard to its correct identity and help to differentiate it from the closely related other species of *Centipeda*.

Keywords: *Centipeda minima*; Pharmacognostic; Bio-chemicals; Ethno-medicinal and Himachal Pradesh.

INTRODUCTION: India is the 8th largest country having a total of around 47 thousand plant species, out of which more than 7,500 species are cited as therapeutic plants. There are many intrinsic factors which direct the growth and therapeutic quality of herbs. This is largely due to change in their chemical constitution which often leads to alter in their bioactivity. Due to these intrinsic uncontrollable variations standardization becomes enormously important. Standardization of natural products is a multipart assignment due to their heterogeneous composition, which is in the form of whole plant, plant parts or their extracts.¹ To ensure reproducible quality of herbal products, proper control of preliminary material is utmost essential. The first step towards ensuring quality of starting material is validation. Plant secondary metabolites represent a tremendous resource for commerce. Biochemists play essential role in the chemical exploration of these plants.² Bio-chemical studies may be aimed at characterizing the chemical makeup of complex plant extracts. Bio-chemical screening can assist taxonomic classification whilst bioassay identifies phyto-chemically active compounds in complex plant extracts and some secondary products perform signaling functions as plant hormones and pheromones.³ Plants produce an incredible array of secondary metabolites and many of these have been developed into economically important products including; oils, gums, resins, tannins, rubber, waxes, pigments, fla-

vors, fragrances, surfactants, preservatives, pesticides and pharmaceuticals.⁴

Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper manage of preliminary material is utmost essential.⁵ The first step towards ensuring quality of preliminary material is authentication. Thus, in recent years there has been a quick increase in the standardization of selected therapeutic plants of potential remedial significance. Despite the modern techniques, identification of plant drugs by pharmacognostic study is more reliable.⁶ According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. The developed method was further validated as per ICH guidelines to indicate its suitability.

This review deals with the isolation, identification and structural elucidation of the phytochemical compounds from *C. minima*. It reveals its anti-inflammatory and antioxidant properties. *C. minima* usually occurs near wet places along banks of dams, creeks and rivers, ditches and it is widespread throughout the temperate regions of Asia, Africa and

Australia. *Centipeda minima* is usually referred to as sneeze weed, old man weed or gukwonderuk.⁷ The review of literature reveals that this herb is under studied in the north-western Himalaya.⁸⁻⁴⁶

MATERIALS AND METHODS:

Plant material: *Centipeda minima* is commonly known as “Chikdoo” in lower shivalik hills. The leaves of *Centipeda minima* were selected for the study. The plant material was authenticated by. The leaves were shade dried, reduced to coarse powder and stored in airtight container till further use.

Physical and pharmacognostic evaluation: The physical values like total ash, acid insoluble ash, water-soluble ash, alcohol soluble extractive and water-soluble extractives were determined. Air-dried powdered material has been subjected to qualitative and quantitative physicochemical estimations.

Ash values: Weighed about 3gm of air dried powdered drug and taken in a silica crucible to incinerate by gradually increasing the temperature to make it dull red hot until free from carbon and then weighed, repeatedly for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.⁴⁷

a) Analysis of acid insoluble ash value: The total ash was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was filtered, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

b) Analysis of water soluble ash value: The total ash obtained was boiled with 25ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at 450°C. The percentage of water soluble was determined with reference to the air dried drug.

Weight loss on drying: Loss on drying is the loss in weight in percent weight by weight indomitable by weighing 1.5gm of powdered drug in a tared porcelain dish and dried at 105°C in hot air oven. The percentage loss of drying to the air dried substance was calculated.

Extractive values of crude drug: Extractive values of crude drugs are functional for their appraisal and these values identify the nature of the constituents present in a crude drug. Analysis of alcohol soluble extractive value 5gm of the air-dried coarse powder of the plant material was macerated with 100ml of 90 percent ethanol in a closed flask for 24 hours, shaking gently during the first 6 hours and allowed to stand for 16 hours. Thereafter, it was been filtered and dried at

105°C and weighed. The percentage of ethanol soluble extractive value was calculated with the air-dried drug. The percentage of water soluble extract was calculated with reference to the air dried drug.

Fluorescence analysis: Fluorescence characteristics of the powdered plant material were analyzed in daylight and UV light.

Extract preparation for biochemical studies: The powdered leaves were kept separately in soxhlet apparatus and again treated with petroleum ether, hydroalcohol (mixture of 70 percent ethanol and 30 percent distilled water) and distilled water. The resultant was distilled in vacuum under low pressure and to remove the solvent completely and later dried in a desiccator.⁴⁸

Qualitative chemical analysis: Qualitative chemical tests have been performed to determine the presence of alkaloids, carbohydrates, cardiac glycosides, polyphenols, saponins and terpenoids.

Alkaloids analysis:

(i) Dragendorff's test: To 1 ml of the extract, added 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(ii) Mayer's test: To 1 ml of the extract, added 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.

Proteins analysis:

Millon's test: 1ml of test solution was acidified with sulphuric acid and mixed Millon's reagent and then, boiled to get yellow precipitates which indicate the presence of protein.

Glycosides analysis:

Keller-Killiani test: 1gm of powdered drug was extracted with 10ml of 70 percent alcohol for 2 minutes, filtered, and to the filtrate added 10ml of water and 0.5ml of strong solution of lead acetate and the filtrate is gently shaken with 5ml of chloroform. The chloroform layer is alienated in a porcelain dish and separates the solvent by gentle evaporation. Dissolved the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5 percent ferric chloride solution. Transferred to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer formed at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

Carbohydrates analysis:

Molisch's test: To 2ml of the extract, added 1ml of α -naphthol solution, added concentrated H_2SO_4 and pur-

ple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates.

Fehling's test: To 1ml of the extract, added equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

Phenolic compounds analysis: The extract was treated with Potassium ferric cyanide and NH_4 solution and a deep red color indicates the presence of tannins.

Flavonoids Analysis:

(i) **Shinoda's test:** The alcoholic extract tested with magnesium foil and concentrated HCl furnish passionate cherry red color which shows the presence of flavonones and orange red colour shows the presence of flavonols.

(ii) The extract treated with NaOH, formation of yellow colour indicates the presence of flavonols.

(iii) The extract treated with concentrated H_2SO_4 , yellow or orange colour shows flavonols.

Steroids analysis:

Salkowski test: Dissolved the extract in chloroform and add equal volume of concentrated H_2SO_4 . Bluish red to cherry colour in chloroform layer as well as green fluorescence in the acid layer indicates the steroidal components.

Fixed oils and fats analysis:

(i) **Spot Test:** Press a small quantity of extracts between the filter paper and oil stains on paper represents the presence of fixed oils.⁴⁹

(ii) **Saponification test:** To 1ml of the extract, add few drops of 0.5 N alcoholic Potassium hydroxide along with a drop of phenolphthalein as well as water bath it for 1-2 hours. The soap formation or partial neutralization of alkali shows the presence of fixed oils and fats.⁵⁰

RESULTS AND DISCUSSION:

Botanical description: *Centipeda minima* (Linn.) A. Br. & Asch. is a herbaceous weed which has worldwide distribution in tropical and sub-tropical regions. It flowers during March to October. The flowers are yellow in coloured. It is an aromatic herb, 8-20cm tall, often much branched, prostrate in habit. Its stem is filiform, ribbed, internodes 2-10mm long, 1 mm large, sparsely to densely covered with fine white, cobwebby hairs. Leaves alternate, simple, narrowly spatulate, 5-20mm \times 1-7mm, base attenuate, apex obtuse and mucronulate, sometimes three-lobed at the apex and

entire at the lower part, margins pinnati-lobed or dentate (lobes or teeth mucronulate), sparsely pilose on both sides; petiole and stipules absent.⁵¹ Inflorescence an axillary head, 2-4mm in diameter. Flowers all tubular, marginal flowers numerous, female, corolla 0.2mm long, pilose, whitish, disk flowers few, bisexual, corolla 0.5mm long, campanulate, deeply 4-lobed, yellow or tinged with violet; anthers 4, 0.4mm long, apically thickened; ovary obconical, 4-angled; style filiform, short, bifid. Fruit an oblong and curved achene, 1mm long, 4-angled, angles with appressed hairs 1 mm long, white, pistil more or less persistent; pappus absent. Seedling with epigeal germination, hypocotyl 2mm long, cotyledons subsessile, elliptical, 1.8mm \times 0.9mm, base attenuate, apex rounded, glabrous, epicotyl absent, first leaves opposite, subsessile, elliptical, mid-vein distinct, base attenuate, margin entire, apex apiculate, glabrous.⁵²

Propagation and Planting: Seed are sown in a seed tray in mid spring and then planted. *Centipeda minima* propagate through achenes, which are zoo- and hydrochorous.

Diseases and pests: It is resistant to root-knot nematodes and other pests.

Harvesting and Post-Harvesting: It is harvested during the time of flower heads blooming and post harvest after cleaning and drying.

Bio-chemical importance: Its oil contains bitter compounds viz; myriogynic acid and myriogynin, several flavonoids viz; quercetin, quercetin-3-methylether and kaempferol-7 rhamnoside, and pentacyclic triterpenes viz; pharmacognostic and fluorescence analysis of leaves has explored the presence of flavonoids, tannins, phenol, alkaloids, glycosides, fats and carbohydrates.⁵³ The chief constituents namely myriogynic acid and myriogynin, several flavonoids including quercetin, quercetin-3-methylether and kaempferol-7 rhamnoside, and pentacyclic triterpenes palmitic acid, phytol, 1,3,5-tri-tertbutyl-benzene, and artemisia ketone present in it.⁵⁴ A methanolic extract of dried aerial parts showed significant activity against herpes simplex virus, polio virus and sindbis virus. An infusion of dried plants exhibited antitussive activity in mice at 0.5g/kg, and the aqueous extract showed antispasmodic activity against acetylcholine- and histamine-induced spasms of guinea-pig ileum.⁵⁵ The ether extract showed anaphylactic activity when administered antiperitoneally to rats. A weak cytotoxic activity of methanol and aqueous extracts was shown in culture of mammary microalveolar cells. The ethyl acetate extract, however, revealed strong cytotoxic activity on HeLaS3 cells, with a IC_{50} value less than 10 $\mu\text{g/ml}$, and the LD_{50} of the ethanol ex-

tract, injected intraperitoneally in mice, at 500-750mg/kg.

Trade information: *Centipeda minima* is used as an ethno-medicinal plant and is not a part of trade chain in the international market.⁵⁶

Geographical distribution: It is cosmopolitan and globally inhabited in Temperate-Asia (China, Guangdong, Hunan, Sichuan, Yunnan, Zhejiang, Eastern Asia, Japan) Tropical-Asia (Nepal, Indo-China, Burma, Thailand, Vietnam, Malaysia, Jawa, Papua New Guinea, Philippines) Australia and Sri Lanka Pacific. It is found in Indian subcontinent (Assam, Bangladesh, Bihar, Maharashtra, Orissa, Punjab, Tamil Nadu, Uttar Pradesh). In Himachal Pradesh, It is frequently found in Una, Hamirpur, Bilaspur, Mandi, Kangra, Solan and Sirmour.⁵⁷

Ethno-medicinal remediation: It is used in mostly against eye and nose infections. The leaves, when squeezed between the fingers and inhaled, make the eyes water, clear the head and provoke sneezing. It is also generally used in cough, common cold and bronchitis disorders. Its decoction is mostly used in paralysis and pain in the joints, and also against malaria, hepatitis, diabetes mellitus, eczema, insect or snake bites, and opium poisoning in lower shivalik hills of Himachal Pradesh. In India, the herb is boiled to a paste and applied to the cheeks for toothache, and also used for other swellings and inflammations. The infusion of leaves is used in ophthalmia as well as the unguent of the grinded herb mixed with honey of applied on the navel of a pregnant-lady, causes abortion.⁵⁸

Pharmacognostical studies: The efficiency and accuracy of pharma products are depend on its extraction by applying standard operating procedure. The physicochemical and bio-chemical characters of *Centipeda minima* were studied and the results are presented in

tables 1. Inorganic materials, such as carbonate, silicates, oxalates, and phosphates have analysed through its total ash-value (5.2 percent). The inorganic components are extracted by heating organic components and release CO₂. The findings of extractive values reveal that it contains higher amount of highly water soluble bio-constituents. The study revealed that the leaves of *Centipeda minima* contain higher amount of semi polar and polar secondary metabolites as well as the pharmacological activity of plant material varies according to its polarity or nature of bio-chemical constituents. Unless specified the dried plant material is ground in a Waring blender and then extracted in the specified solvents by steeping overnight at room temperature. The resulting extracts are then filtered through a glass frit and evaporated to dryness on a rotary evaporator. The preliminary screening of each plant is performed by LC-MS. MilliQ (MQ) water, Liquid Chromatography-Mass Spectrometry (LC-MS) and bio-chemicals screening are employed throughout the course of this research. This plant has potential to produce bio-chemicals as Carbohydrates, Protein and Lipids that are utilized as food by herbivores, and also biosynthesize multitude of compounds like Glycosides, Alkaloids, Volatile oils, Tannins etc., that exerts a physiologic effect. The compounds that are responsible for diagnostic effect are usually the secondary metabolites.

Exploration of a crude drug embraces through consideration of both primary and secondary metabolites obtained as a result of plant metabolism can be subjected to preliminary bio-chemical screening for the detection of various plant constituents. Bio-chemical screening reveals that the petroleum ether extract from its leaves indicate the presence of fats and oils, alkaloids, polyphenol, glycosides, flavonoids (Table 1).^{59,60,61}

Table: 1 Bio-chemicals present in the leaves of *Centipeda minima* Linn.

Bio-chemicals	Analytical Tests	Petroleum Ether	HCl	Aqueous
Alkaloids	Dragendorff's test	-	+	-
	Mayer's test	-	-	-
Proteins analysis	Millon's test	-	-	+
Glycosides analysis	Keller-Killiani test	-	+	+
Carbohydrates analysis	Molisch's test	-	+	+
	Fehling's test	-	+	+
Flavonoids Analysis	Shinoda's test	-	+	+
Steroids analysis	Salkowski test	-	-	-
Fixed oils and fats analysis	Spot Test	+	-	-
	Saponification test	+	-	-

CONCLUSION: The systematic study on *Centipeda minima* shows its ethnomedicinal and pharmacognostic importance as well as alternative for primary health

care system in lower shivalik hill regions of Himachal Pradesh. The natural stock of medicinal plants is under tremendous pressure, since, must be conserved

and promoted its commercial cultivation. Pharmacognostic and bio-chemical investigation also provides useful information in regard to its correct identity and help to differentiate it from the closely related other species of *Centipeda*.

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CONFLICT OF INTEREST: Authors declares that there is not any conflict of interest.

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