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PHYTOCHEMICAL AND ANTIEPILEPTIC EVALAUATION DIFFERENT EXTRACTS OF CENTELLA ASIATICA (GOTU KOLA)

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ABSTRACT

Centella asiatica, commonly known as "Gotukola" is naturally used in the treatment of different diseases, for example epilepsy. The active principles of the leaves of plant were extracted into different solvents, Methanol, Water, n-Hexane, Chloroform, Ethyl acetate and n-Butanol. Powdered plant material was soaked in methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Rotovapour yielding a gum-like residue, which was then suspended in water and extracted with various organic solvents of increasing polarity. The extracts were freeze dried and were used for phytochemical screening techniques. This analysis reveals that the extracts of *Centella asiatica* has rich in Reducing sugars, Anthraquinones, Saponins, Flavonoids, Terpenoids, Tannins, Alkaloids, Resins, Aldehydes & Ketones and Phenols, which are popular phytochemical constituents.

Key words: *Centella asiatica*, Epilepsy, Phytochemical screening.

INTRODUCTION

Medicinal plants have contributed immensely to health care in India. This is due in part to the recognition of the value of traditional medical systems, particularly in Asian origin, and the identification of medicinal plant from indigenous pharmacopoeias, which have significant healing power.

Among all families of the plant kingdom, members of the Apiaceae (Umbelliferae) have been used for centuries in folk medicine. *Centella asiatica*, commonly known as "Gotu kola" is naturally used in the treatment of different diseases, for example epilepsy. It also showed effect against herpes simplex viruses [1], *Mycobacterium leprae* and *Mycobacterium tuberculosis* [2] and antidepressant activity in rats [3]. *Centella asiatica* is referred as one of the great multipurpose miracle herbs of oriental medicine. It is considered as one of the most powerful rejuvenating herbs in Indian Ayurvedic medicine. It has been used in ayurvedic preparation either in the fresh or in the extract form [4]. CA has been subjected to extensive experimental and clinical investigation [5]. The active constituents of *Centella* include triterpenoid glycosides (asiatic acid, asiaticoside, madecassic acid, madecassoside, oxyasiaticoside, and centelloside) [6,7]; saponin glycosides (1.4-3.4%) (brahmiside, brahminoside); flavonol glycosides (quercetin-3-glycoside and Kampferol-3-glycoside); flavonoids viz., naringin, quercitin, rutin, cathecin, kampeferol and apigenin; phytosterols such as ß-sitosterol, stigmasterol and camposterol and a volatile oil consisting of vallerin, camphor, cineole and terpene acetate that comprises 35% of the total oil content. CA also contains naturally occurring vitamins A, B, C, G, K, tannins (24.5%); essential oils (0.8-1%); monoterpenes (α-pinene, ß-pinene, myrcene, α-terpineol, borneol); sesquiterpenes (α-copanene, ß-elemene, ß-caryophyllene, trans-ß-farnesene, germacrene, bicyloelemene); several aminoacids (lysine, alanine, phenylalanine, serine, aspartic acid, glutamic acid); fatty acids (palmitic, oleic and linoleic acids); resin (8.9%); an alkaloid named hydrocotyline and elements Calcium, Magnesium and Sodium. CA appears to be non-toxic but mild allergic causes contact dermatitis in

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sensitive individuals [8].

This study was designed to evaluate the active constituents of different extracts of *Centella asiatica* and to determine the presence of secondary metabolites.

Reducing sugars

A reducing sugar is any sugar that, in an alkaline solution, forms some aldehyde or ketone. This allows the sugar to act as a reducing agent, for example in the Maillard reaction and Benedict's reaction. Reducing sugars include glucose, fructose, glyceraldehyde, lactose, arabinose and maltose. All monosaccharides which contain ketone groups are known as ketoses, and those which contain aldehyde groups are known as aldoses. Significantly, sucrose and trehalose are not reducing sugars.

Anthraquinones

Anthraquinone (9,10-dioxoanthracene) is an aromatic organic compound. It is a derivative of anthracene. It has the appearance of yellow or light gray to gray-green solid crystalline powder. Anthraquinones naturally occur in some plants (eg. aloe latex, senna, rhubarb, and Cascara buckthorn), fungi, lichens, and insects, where they serve as a basic skeleton for their pigments. Natural anthraquinone derivatives tend to have laxative effects.

Anthraquinone is used in production of dyes, such as alizarin. Many natural pigments are derivatives of anthraquinone. Anthraquinone may be used as a catalyst in production of chemical pulp by the Kraft or Soda-AQ processes. Another use is as a bird repellant on seeds. A derivative of anthraquinone called 2-ethylanthraquinone is used to produce hydrogen peroxide commercially.

Saponins

Saponins are a class of chemical compounds, one of many secondary metabolites found in natural sources, with saponins found in particular abundance in various plant species. Specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by their being composed of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. A ready and therapeutically relevant example is the cardio-active agent digoxin, from common foxglove.

There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals. There is evidence of the presence of saponins in traditional medicine preparations, where oral administrations might be expected to lead to hydrolysis of glycoside from terpenoid (and obviation of any toxicity associated with the intact molecule). But as is often the case with wide-ranging commercial therapeutic claims for natural products. The claims for organismal/human benefit are often based on very preliminary biochemical or cell biological studies and mention is generally omitted of the possibilities of individual chemical sensitivity, or to the general toxicity of specific agents and high toxicity of selected cases.

Flavonoids

Flavonoids (or bioflavonoids), also collectively known as Vitamin P and citrin, are a class of plant secondary metabolites. According to the IUPAC nomenclature they can be classified into three.

1. Flavonoids, derived from 2-phenylchromen-4-one (2-phenyl-1,4-benzopyrone) structure (examples: quercetin, rutin).
2. Isoflavonoids, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure.
3. Neoflavonoids, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure.

The three flavonoid classes above are all ketone-containing compounds, and as such, are flavonoids and flavonols. This class was the first to be termed "bioflavonoids." The terms flavonoid and bioflavonoid have also been more loosely used to describe non-ketone polyhydroxy polyphenol compounds which are more specifically termed flavanoids, flavan-3-ols, or catechins (although catechins are actually a subgroup of flavanoids).

Flavonoids (both flavonols and flavanols) are most commonly known for their antioxidant activity. Flavonoids (specifically flavanoids such as the catechins) are "the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants". Flavonols, the original bioflavonoids such as quercetin, are also found ubiquitously, but in lesser quantities. Both sets of compounds have evidence of health-modulating effects in animals which eat them.

Terpenoids

Terpenoids (or isoprenoids), a subclass of the prenyllipids (terpenes, prenylquinones, and sterols), represent the oldest group of small molecular products synthesized by plants and are probably the most widespread group of natural products. Terpenoids can be described as modified terpenes, where methyl groups are moved or removed, or oxygen atoms added. Inversely, some authors use the term "terpenes" more broadly, to include the terpenoids. During the 19th century, chemical works on turpentine led to name "terpene" the hydrocarbons with the general formula $C_{10}H_{16}$ found in that complex plant product. These terpenes are frequently found in plant essential oils which contain the "Quinta essentia", the plant fragrance. They are universally present in small amounts in living organisms, where they play numerous vital roles in plant physiology as well as important functions in all cellular membranes. Terpenoids are extraordinarily diverse but they all originate through the condensation of the universal phosphorylated

derivative of hemiterpene, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) giving geranyl pyrophosphate (GPP). In higher plants, IPP is derived from the classic mevalonic acid pathway in the cytosol but from the methylerythritol phosphate pathway in plastids. It is generally accepted that the cytosolic pool of IPP serves as a precursor of sesquiterpenes, triterpenes, sterols and polyterpenes whereas the plastid pool of IPP provides the precursors of mono-, di- and tetraterpenes.

Tannins

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine. Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruit and the aging of wine. The term tannin (from tanna, an Old High German word for oak or for tree) refers to the use of wood tannins from oak in tanning animal hides into leather; however, the term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. The compounds are widely distributed in many species of plants, where they play a role in protection from predation and perhaps also in growth regulation.

Alkaloids

The basic unit in the biogenesis of the true alkaloids are amino acids. The non-nitrogen containing rings or side chains are derived from terpene units and / or acetate, while methionine is responsible for the addition of methyl groups to nitrogen atoms. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (potassiomercuric iodide solution). A common property of tropane alkaloids is a methylated nitrogen atom N-CH3 at one end of the molecule. This chemical structure is also found in the neurotransmitter acetylcholine, which transmits impulses between nerves in the brain and neuromuscular junctions. The anesthetic properties of tropane alkaloids may relate to their interference with acetylcholine, perhaps by competing with it at the synaptic junctions, thus blocking or inhibiting nerve impulses. It is interesting to note that the infamous tropane alkaloid, cocaine, is also a local anesthetic when injected into skin or muscle tissue. This property led to the discovery and synthesis of the more potent compound, novocain, widely used in dentistry.

There are three main types of alkaloids: Figure - 1

Resins

Resin is a hydrocarbon secretion of many plants, particularly coniferous trees. It is valued for its chemical constituents and uses, such as varnishes and adhesives, as an important source of raw materials for organic synthesis, or for incense and perfume. Fossilized resins are the source of amber. Resins are also a material in nail polish.

The term is also used for synthetic substances of similar properties. Resins have a very long history and are mentioned by both ancient Greek Theophrastus and ancient Roman Pliny the Elder, especially as the forms known as frankincense and myrrh. They were highly prized substances used for many purposes, especially perfumery and as incense in religious rites.

However, resins consist primarily of secondary metabolites or compounds that apparently play no role in the primary physiology of a plant. While some scientists view resins only as waste products, their protective benefits to the plant are widely documented. The toxic resinous compounds may confound a wide range of herbivores, insects, and pathogens; while the volatile phenolic compounds may attract benefactors such as parasitoids or predators of the herbivores that attack the plant

Aldehydes and Ketones

Aldehydes and ketones are simple compounds which contain a carbonyl group - a carbon-oxygen double bond. They are simple in the sense that they don't have other reactive groups like -OH or -Cl attached directly to the carbon atom in the carbonyl group - as you might find, for example, in carboxylic acids containing -COOH.

An aldehyde is an organic compound containing a terminal carbonyl group. This functional group, which consists of a carbon atom bonded to a hydrogen atom and double-bonded to an oxygen atom (chemical formula O=CH-), is called the aldehyde group. The aldehyde group is also called the formyl or methanoyl group.

The word aldehyde seems to have arisen from alcohol dehydrogenated. In the past, aldehydes were sometimes named after the corresponding alcohols, for example, vinous aldehyde for acetaldehyde. (Vinous is from Latin vinum = wine (the traditional source of ethanol), cognate with vinyl.

The aldehyde group is polar. Oxygen, more electronegative than carbon, pulls the electrons in the carbon-oxygen bond towards itself, creating an electron deficiency at the carbon atom.

Owing to resonance stabilization of the conjugate base, an α-hydrogen in an aldehyde is more acidic than a hydrogen atom in an alkane, with a typical pKa of 17.

In organic chemistry, a ketone is a type of compound that features one carbonyl group (C=O) bonded to two other carbon atoms, i.e., R3CCO-CR3 where R can be a variety of atoms and groups of atoms. With carbonyl carbon bonded to two carbon atoms, ketones are distinct from many other functional groups, such as carboxylic acids, aldehydes, esters, amides, and other oxygen-containing compounds. The double-bond of the carbonyl group distinguishes ketones from alcohols and ethers.

A carbon atom adjacent to a carbonyl group is called an α-carbon. Hydrogen atoms attached to these α-carbon centers are called α-hydrogens. Ketones with α-hydrogen centers participate in a so-called keto-enol tautomerism. The reaction with a strong base gives the corresponding enolate, often by deprotonation of the enol.

Phenols

In organic chemistry, phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol (C6H5OH). Although similar to alcohols, phenols have unique properties and are not classified as alcohols (since the hydroxyl group is not bonded to a saturated carbon atom). They have higher acidities due to the aromatic ring's tight coupling with the oxygen and a relatively loose bond between the oxygen and hydrogen. The acidity of the hydroxyl group in phenols is commonly intermediate between that of aliphatic alcohols and carboxylic acids (their pKa is usually between 10 and 12). Loss of a positive hydrogen ion (H^+) from the hydroxyl group of a phenol forms a corresponding negative phenolate ion or phenoxide ion, and the corresponding salts are called phenolates or phenoxides. Phenols can have two or more hydroxy groups bonded to the aromatic ring(s) in the same molecule. The simplest examples are the three benzenediols, each having two hydroxy groups on a benzene ring. Some phenols are germicidal and are used in formulating disinfectants. Others possess estrogenic or endocrine disrupting activity.

MATERIALS AND METHODS

Phytochemical screening

Phytochemicals with biological activity have great utility as pharmaceuticals. The lyophilized extracts of CA such as Crude, Methanol (ME), n-Hexane extract (n-HE), Chloroform extract (CE), Ethyl acetate extract (EAE), n-Butanol extract (n-BE) and Aqueous extract (AE) were subjected to various chemical tests in order to determine the secondary plant constituents present by employing various methods as follows:

Test for the presence of Reducing Sugars

To 2 ml of the extract, 5ml of a mixture (1:1) of Fehling's solution I (A) and Fehling's solution II (B) were added and the mixture boiled in a waterbath for five minutes. A brick-red precipitate indicated the presence of free reducing sugars [9].

Test for the presence of Anthraquinones

0.5g of the extract was taken with 10 ml of benzene, filtered and 5 ml of 10 % ammonia solution added to the filtrate. The mixture was shaken, the presence of a pink, red or violet colour in the ammonical (lower) phase indicated the presence of Anthraquinones [10].

Test for the presence of Saponins

0.5g of the extract was dissolved in a 10 ml of distilled water in a test-tube, the test tube was stopperred with a cork and shaken vigorously for 30 seconds and then allowed to stand for 45 minutes. The appearance of frothing which persists on warming indicated the presence of Saponins [10].

Test for the presence of Flavonoids

To a portion of the dissolved extract, a few drops of 10 % ferric chloride solution were added. A green or blue colour indicated the presence of phenolic nucleus [9].

Test for the presence of Steroids/Terpenes

0.5g of the extract was dissolved and 2 ml of acetic anhydride and cooled well in ice. Sulphuric acid was then carefully added. A colour change from violet to blue to green indicated the presence of a steroidal nucleus [10].

Test for the presence of Tannins

0.5 g of the extract was dissolved in 5 ml of water followed by a few drops of 10 % ferric chloride. A blue-black, green, or blue-green precipitate would indicate the presence of tannins [10].

Test for the presence of Alkaloids

0.5g of ethanol extract was stirred with 5ml of 1 % aqueous hydrochloric acid on a steam-bath; 1 ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated with Dragendorff's reagent. Turbidity or precipitation with either of these reagents would indicate the presence of alkaloids in the extracts [10].

Test for the presence of Resins

10 ml of petroleum ether extract was obtained in a test-tube, the same amount of copper acetate solution was added and the mixture was shaken vigorously and allowed to separate. A green colour indicates the presence of resin [9].

Test for the presence of Aldehydes and Ketones

To 30 mg of CA extract 2 ml of 95% ethanol and 3 ml of 2,4-dinitrophenylhydrazine reagent were added. It was allowed to stand for 15 minutes. The presence of precipitate is the positive test for aldehydes and ketones. [11]

Test for the presence of Phenols

15 mg of the CA extracts were suspended in 0.5 ml of methylene chloride and 3-5 drops of a 1% solution ferric chloride prepared in methylene chloride were added. A drop of pyridine was added and stirred. It will produce red wine color if phenols were present. [12].

RESULTS AND DISCUSSION

Phytochemical screening of the plant, *Centella asiatica*, showed that the plant is rich in reducing sugars, anthraquinones, saponins, flavanoids, terpenoids, tannins, alkaloids, resins, aldehydes & ketones and phenols. However, tannins, alkaloids and phenols were absent in n-Hexane extract whereas resins were absent in methanol extract. Tannins and phenols were also absent in chloroform extract. The ethyl acetate extract an aqueous extract are devoid of alkaloids and resins. Similarly resins were absent in ethyl acetate, n-Butanol and aqueous extracts. Aldehydes and ketones were absent in n-Butanol and aqueous extracts.

Although bioassay guided fractionation was not performed in the present study, the preliminary results indicate the presence of different bioactive factors in the extracts of CA. The presence of flavanoids and tannins in this plant extract is likely to be responsible for the free radical scavenging activity [13]. In addition, the Flavonoids may also be associated with different pharmacological properties such as anti-inflammatory [14], antipyretic, analgesic and spaspholytic activities [15,16]. The presence of tannins also suggests its ability to play a major role in antidiarrhea and antihaemorrhagic activities [17].

The presence of saponins in all the extracts reveals that these compounds have imminse significance as anti-hyper cholesterol, hypotensive and cardiac depressant properties [18,19]. It has been reported that saponins have been found to be potentially useful for the treatment of hyperglycemia [20] and anti-inflammation [14].

The plant is also a very good source of terpenoids which play an important role in wound and smear healing [21. The pharmacological studies showed that terpenoids strengthen the skin, increases the antioxidant levels in wounds and thus prevents any oxidative damage caused due to inflammation [21]. The terpenoids can also be used as a rejuvenating agent and hence, the extracts of CA have been found to be a very useful remedy for anti-aging and overall health enhancement [22]. In addition, the terpenoids can be used in reducing blood pressure in patients with high blood pressure and diabetes [21].

It was also found that alkaloids were only present in methanolic, chloroform and n-Butanol extracts. Which have clinical significance in the treatment of hypertension [23,24]. Clinical studies have shown that the different extracts of *Centella asiatica* cause sedative effect; increased mean RBC count, haemoglobin concentration, blood sugar, serum cholesterol, total serum protein in adults [25].

Extracts of *Centella asiatica* have also successfully treated in surgical wounds, skin grafts, gangrene, and traumatic injuries [26], chronic skin lesions and leprosy wounds [27]. *Centella asiatica* showed wound healing activity [28-30] antitumor activity [31] anti-anxiety activity[32] anti-hepatoma activity [33] cognition-enhancement in rats [34,35].

Centella asiatica was effective in improving microcirculation in venous hypertension and diabetic microangiopathy [36]. It was also used in the treatment of tuberculosis, syphilis, amoebic dysentery and common cold, also known as anti-aging plant [37]. Somchit reported antinociceptive and anti-inflammatory effects of *Centella asiatica*[38]. The ethanolic extract of *Centella asiatica* significantly increased the wound breaking strength in animals [39]. Triterpenes such as asiatic acid, madecassic acid and asiaticoside extracted from *Centella asiatica* stimulate extracellular matrix accumulation in the way of wound healing [40]. Aqueous extract of *Centella asiatica* showed faster gel formation on open wounds when compared to ointment and cream formulation [41]. Pre-co-treatment with aqueous extract of *Centella asiatica* effectively counteracted the cardiotoxicity caused due to the disorganization of mitochondrial structure and systolic failure [42].

Total triterpenic fraction of *Centella asiatica* showed a combined improvement of the microcirculation and capillary permeability in patients with venous hypertension [43]. Quantitative analysis of C. asiatica leaf revealed high concentration of total phenolic compounds, in particular, catechin, quercetin, and rutin [44]. *Centella asiatica* (CA) is a tropical medicinal plant enriched with bioflavonoids, triterpenes and selenium and reported to rejuvenate the cells and promote physical and mental health [45].

These phytochemical screening on qualitative analysis shows CA has rich in Reducing sugars, Anthraquinones, Saponins, Flavonoids, Terpenoids, Tannins, Alkaloids, Resins, Aldehydes & Ketones and Phenols, which are popular phytochemical constituents.

Figure 1. Types of Alkaloids

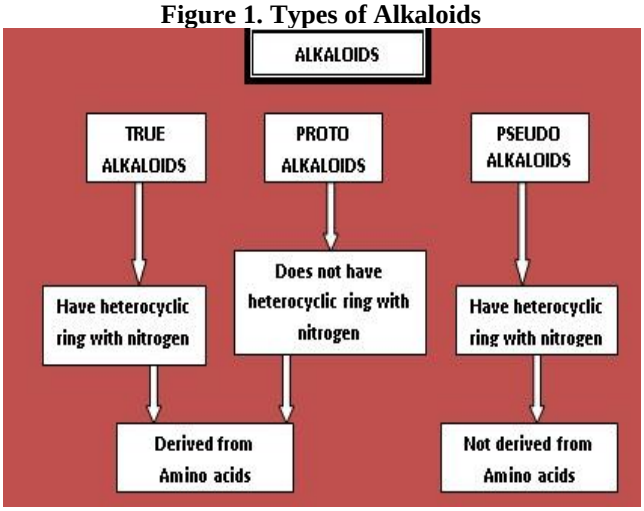

Table 1. Qualitative analysis of phytochemical constituents, in different extracts of *Centella asiatica*

S. No	Name of the Phytochemical	Crude extract	ME	n-HE	CE	EAE	n-BE	AE
1.	Reducing sugars	+	+	+	+	+	+	+
2.	Anthraquinones	+	+	+	+	+	+	+
3.	Saponins	+	+	+	+	+	+	+
4.	Flavonoids	+	+	+	+	+	+	+
5.	Terpenoids	+	+	+	+	+	+	+
6.	Tannins	+	+	—	—	+	+	+
7.	Alkaloids	+	+	—	+	—	+	—
8.	Resins	+	—	+	+	—	—	—
9.	Aldehydes and Ketones	+	+	+	+	+	—	—
10.	Phenols	+	+	—	—	+	—	+

+ Present of phytochemical, — Absent of phytochemical

CONCLUSION

This study may be useful in the area of pharmacology and synthesis of new antiepileptic medicines. Centella asiatica extracts or the bioactive compounds present in the extracts may be beneficial in the antiepileptic treatment. Hence, the information gained from the present study can be used for proposing a better pharmacological utensil to treat the epilepsy and related disorders. The present study also helps in the discovery of new neuroprotective components from the medicinal plants.

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