

Comparative Evaluation of Phytochemical Constituents in *Centella asiatica* and *Lawsonia inermis*

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ABSTRACT

Medicinal plants are important sources for isolation of pharmaceutical drugs. The current available drugs are in many ways either inefficient or unaffordable to ever increasing forms of microbial infections. Screenings of medicinal plants used by the traditional medicinal healers are the main sources for formulation of herbal drugs. Phytochemicals present in plants are economically important sources of drugs, fragrances, pigments, food additives and pesticides *Lawsonia inermis* has maximum contents of tannin. The present study was carried out to comparatively evaluate the phytochemical constituents of *Centella asiatica* and *Lawsonia inermis* using methanolic and ethanolic extracts, respectively. Qualitative phytochemical screening was performed using standard chemical tests to identify alkaloids, flavonoids, tannins, saponins, glycosides, carbohydrates, phenolic compounds, and terpenoids. Alkaloids were detected using Dragendorff's, Mayer's, and Wagner's tests, while flavonoids were confirmed by Shinoda, zinc–hydrochloride reduction, and lead acetate tests. The study provides scientific support for the traditional medicinal uses of these plants and suggests their potential for further pharmacological investigation.

Keywords: *Phytochemical screening, Centella asiatica, Lawsonia inermis, medicinal plants, qualitative analysis.*

INTRODUCTION

The global health problem has been changed into major concern by these bacteria, some of which are resistant to a range of drugs that are now popularly available. The related sections about resistance levels in *Staphylococcus aureus* and *Escherichia coli* offer evidence of this fact [1, 2]. Such difficulties have helped researchers to consider that plants which are primarily medicinal or that contain antibacterial substances may even prove bases for drugs. We can therefore say with some confidence that at least some effects are due to the presence of these compounds in medicinal plants [3]. Medicinal plants not only have direct therapeutic properties, but also can provide lead compounds for drug synthesis [4]. The unique bioactivity of many medicinal plants is believed to come from alkaloids, flavonoids, phenolic compounds, tannins, terpenoids (mostly mono- and sesquiterpenes) and glycosides [5]. They are physiologically active and many [6] have been developed into pharmaceuticals, food additives, perfumes or pesticides. Many plant natural bioactive molecules are aromatic or saturated organic compounds and are acquired through extraction using polar solvents such as ethanol or methanol [7]. Therefore, carrying out phytochemical screening with suitable solvents provides an important initial step in identifying biologically active ingredients. *Centella asiatica* is a medicinal plant naturally occurring in Asian countries and has been used extensively for a long time to treat various diseases such as wounds, bronchitis, asthma, diabetes, kidney disorders, liver complaints, hypertension, and to improve brain functions [8]. The herb is also believed to exhibit neuroprotective and antioxidant potential [9]. Henna, *Lawsonia inermis*, is a medicinal plant reported to possess antibacterial, antifungal and dermatological activities. It has been traditionally used for the treatment of skin diseases, bronchitis, rheumatism, dysmenorrhoea and inflammatory conditions [10]. Since past reports on *C. asiatica* and *L. inermis* have shown them to be potential medicinal herbs, a comparative phytochemical evaluation of these plants is scientifically justified.

MATERIALS AND METHODS

Collection of plant material

Fresh and healthy plant materials of *Centella asiatica* and *Lawsonia inermis* were collected from the local area. The plants were identified and authenticated based on standard taxonomic keys and relevant floras. leaves free from disease and physical damage were selected for further use.

Processing of plant samples

The collected leaves were washed under tap water to remove dust and soil particles, followed by rinsing with distilled water. The cleaned leaves were shade-dried at room temperature and then oven-dried at 35–40 °C for 3–4 days. The dried leaves were pulverized using a sterile electric grinder to obtain a fine powder. The powdered samples were stored in airtight glass containers and protected from light and moisture until extraction.

Preparation of solvent extracts

Ethanolic extract of *Lawsonia inermis*

Five grams (5 g) of powdered leaves of *Lawsonia inermis* were soaked in 25 ml ethanol. The mixture was kept under incubation for 2 days. After extraction, the suspension was filtered using Whatman filter paper. The filtrate obtained was considered the crude ethanolic extract and stored at 4 °C for further analysis.

Methanolic extract of *Centella asiatica*

Five grams (5 g) of powdered leaves of *Centella asiatica* were extracted with 25ml methanol. The mixture was kept under incubation for 2 days. After extraction, the mixture was filtered using Whatman filter paper to obtain the crude methanolic extract, which was stored at 4 °C until use.

TESTS PERFORMED

A. Detection of alkaloids

1) Dragendorff's/ Kraut's test

Few mL filtrate + 1-2 mL Dragendorff's reagents

Observation = The formation of a reddish-brown precipitate indicated a positive result for the presence of alkaloids, whereas the absence of precipitate indicated a negative result [11, 12].

2) Mayer's/ Bertrand's/ Valser's test

Few mL filtrate + 1-2 drops of Mayer's reagent (Along the sides of test tube)

Observation = The formation of a creamy white to yellow precipitate was considered as a positive result, whereas the absence of precipitate indicated a negative result [13, 14].

3) Wagner's test

Few mL filtrate + 1-2 drops of Wagner's reagent (Along the sides of test tube)

Observation = The formation of a brown to reddish precipitate was considered as a positive result, whereas the absence of precipitate indicated a negative result [13, 12].

4) Iodine Test

3mL extract solution + few drops of iodine solution

Observation = The appearance of a blue colour, which disappeared upon boiling and reappeared on cooling, was considered a positive result, whereas the absence of this colour change indicated a negative result [15, 16].

5) Tannic acid test

Acidified extract + 10% tannic acid solution

Observation = The formation of a buff-coloured precipitate was considered a positive result, whereas the absence of precipitate indicated a negative result [17].

B. Detection of Glycosides

1) 10% NaOH test

1mL dil. H_2SO_4 + 0.2mL extract + boiled for 15min. + allowed cooling + neutralize with 10% NaOH + 0.2mL Fehling's solution A & B

Observation = The formation of a brick-red precipitate was considered a positive result, whereas the absence of precipitate indicated a negative result [12].

2) Concentrate H_2SO_4 test

5mL plant extract + 2mL glacial acetic acid + a drop of 5% $FeCl_3$ + conc. H_2SO_4

Observation = The appearance of a brown ring at the interface indicated a positive reaction, while no ring formation was taken as a negative result [17].

C. Detection of Flavonoids

1) Alkaline reagent test

1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl) An intense yellow colour, becomes colourless on addition of diluted acid Plant extract + 10% ammonium hydroxide sol.

Observation = The appearance of yellow fluorescence was considered a positive result, whereas the absence of fluorescence indicated a negative result [18,19].

2) Lead acetate test

1mL plant extract + few drops of 10% lead acetate solution

Observation = The formation of a yellow precipitate was considered a positive result, whereas the absence of precipitate indicated a negative result [14].

3) Shinoda's test/ Mg-hydrochloride reduction test

Plant extract is dissolved in 5mL alcohol + Fragments of magnesium ribbon + few drops of conc. HCl

Observation = The development of a pink to crimson-coloured solution was considered a positive result, whereas the absence of colour change indicated a negative result [20].

4) Shibata's reaction/ Cyanidin test

1gm Aq. extract + dissolved in 1-2 mL 50% methanol by heating + metal magnesium + 5-6 drops of conc. HCl

Observation = The appearance of a red colour was considered a positive result, whereas the absence of colour change indicated a negative result [21].

D. Detection of Phenolics compound

1) Ellagic Acid Test

Plant extract aqueous solution + 5% glacial acetic acid + 5% sodium nitrite solution

Observation = The solution turning muddy with the formation of a Niger brown precipitate was considered a positive result, whereas the absence of such changes indicated a negative result [15].

2) Potassium dichromate test

Plant extract + few drops of potassium dichromate solution

Observation = The development of a dark colour was considered a positive result, whereas the absence of colour change indicated a negative result [22].

E. Detection of Tannin

1) Braymer's test

1mL filtrated + 3mL distilled water + 3 drops 10% Ferric chloride solution

Observation = The development of a blue-green colour was considered a positive result, whereas the absence of colour change indicated a negative result [23].

F. Detection of Phlobatannins

1) HCl test

2mL aq. extract + 2mL 1% HCl (boiled)

Observation = The appearance of a red precipitate indicated a positive reaction, while no precipitate formation was taken as a negative result [24].

G. Detection of Saponins

1) Foam test

0.5gm plant extract + 2mL water (vigorously shaken) Persistent foam for 10 min.

Observation = The formation of a stable foam layer measuring approximately 2 cm in height was considered a positive result, whereas the absence of foam formation indicated a negative result [13].

2) NaHCO₃ test

Plant extract + few mL sodium bicarbonate solution + distilled water (vigorously shaken)

Observation = The formation of a stable, honeycomb-like froth was considered a positive result, whereas the absence of froth formation indicated a negative result [17].

3) Olive oil test

Aq. extract + 5mL distilled water; shaken vigorously + few drops of olive oil + shaken vigorously

Observation = The appearance of foam was considered a positive result, whereas the absence of foam formation indicated a negative result [25].

H. Detection of Diterpenes

1) Copper acetate test

Plant extract is dissolved in distilled water + 3-4 drops of copper acetate solution

Observation = The development of an emerald green colour was considered a positive result, whereas the absence of colour change indicated a negative result [26].

I. Detection of Quinones

1) Sulphuric acid test

10mg extract + dissolved in isopropyl alcohol + a drop of conc. H_2SO_4

Observation = The appearance of a red colour was considered a positive result, whereas the absence of colour change indicated a negative result [27].

J. Detection of Leuconthocyanins

1) Isoamyl alcohol test

5mL plant extract + 5mL isoamyl alcohol

Observation = The development of a red-coloured upper layer indicated a positive reaction, while no colour change in the upper layer was taken as a negative result [28].

K. Detection of Carboxylic acid

1) Effervescence test

1mL plant extract + 1mL sodium bicarbonate solution

Observation = The appearance of effervescence was considered a positive result, whereas the absence of effervescence indicated a negative result [24].

L. Detection of Carbohydrates

1) Molish's test

2mL filtrate + 2 drops of alcoholic α -naphthol + 1mL conc. H_2SO_4 (along the sides of test tube)

Observation = The formation of a violet ring was considered a positive result, whereas the absence of ring formation indicated a negative result [13].

2) Test for starch

Aqueous extract + 5mL 5% KOH solution

Observation = The development of a cinary colouration was considered a positive result, whereas the absence of colour change indicated a negative result[29].

M .Detection of Reducing sugars

1) Benedict's test

0.5mL filtrateb + 0.5mL Benedict's reagent + Boiled for 2 min.

Observation = The development of a green, yellow, or red colour was considered a positive result, whereas the absence of any colour change indicated a negative result [13].

2) Fehling's test

1mL each of Fehling's solution A & B + 1mL filtrateb + boiled in water bath

Observation = The formation of a red precipitate was considered a positive result, whereas the absence of precipitate indicated a negative result [14].

N. Detection of Proteins and Amino acids

1)Ninhydrin test

2mL filtrate + 2 drops of Ninhydrin solution (10mg ninhydrin + 200mL acetone)

Observation = The formation of A purple coloured sol. was considered a positive result, whereas the absence of purple coloured sol indicated a negative result [13].

2) Xanthoproteic test

Plant extract + Few drops of conc. Nitric acid

Observation = The formation of a yellow coloured sol. was considered a positive result, whereas the absence of a yellow coloured sol indicated a negative result [11].

RESULT AND DISCUSSION

A. Detection of Alkaloids		
Test	<i>Lawsonia inermis</i>	<i>Centella asiatica</i>
1) Dragendroff's/ Kraut's test	+	+
2) Mayer's/ Bertrand's test	-	-
3) Wagner's test	+	+
4) Iodine Test	-	-
5) Tannic acid test	-	+

B. Detection of Glycosides		
1) 10% NaOH test	+	+
2) Concentrate H ₂ SO ₄ test	+	-
C. Detection of Flavonoids		
1) Alkaline reagent test	+	+
2) Lead acetate test	+	+
3) Shinoda's test/ Mg-hydrochloride reduction test	-	+
4) Shibata's reaction/ Cyanidin test	-	-
D. Detection of Phenolics compound		
1) Ellagic Acid Test	-	-
2) Potassium dichromate test	+	+
E. Detection of Tannin		
1) Braymer's test	+	+
F. Detection of Phlobatannins		
1) HCl test	-	-
G. Detection of Saponins		
1) Foam test	-	-
2) NaHCO ₃ test	-	-
3) Olive oil test	-	-
H. Detection of Diterpenes		
1) Copper acetate test	+	-
I. Detection of Quinones		
1) Sulphuric acid test	-	-
J. Detection of Leuconthocyanins		
1) Isoamyl alcohol test	+	-
K. Detection of Carboxylic acid		
1) Effervescence test	-	-
L. Detection of Carbohydrates		
1) Molish's test	-	-
2) Test for starch	+	-
M. Detection of Reducing sugars		
1) Benedict's test	+	-

2) Fehling's test	+	+
N. Detection of Proteins and Amino acids		
1) Ninhydrin test	-	-
2) Xanthoproteic test	-	-

(+ = Present, - = Absent)

The majority of the major secondary metabolites were found in *Centella asiatica*, according to the current phytochemical analysis. Tannins and flavonoids were found in *Centella asiatica*, supporting the findings of previous analyses by [30,31] but in contrast to [32] who reported that these compounds were not present in *Centella asiatica*. Although it contrasts with that of the presence of saponins in this analysis also supports that of [30,31]. Other than these, every result is consistent with previous analyses. Our investigation does not include terpenoids like steroids and saponins or phenols like quinones. Since secondary metabolites are created at particular stages of a plant's life cycle, the aforementioned discrepancies in the phytochemical analysis observations may be the result of different sample collecting times. Plants' phases of growth may even have an impact on the accumulation of secondary metabolites. For the majority of plants, metabolite accumulation peaks during flowering and decreases throughout fruiting. The quality and amount of the secondary metabolites can therefore be affected by the time of harvest or field collection of the samples. Other elements including soil, light, water, temperature, and the plant's nutrient conditions can also have an impact on the characteristics of phytochemical substances found in plants. The physical characteristics and phytochemical components of plant samples can also be influenced by the techniques employed for wild sample collecting, growing, harvest, post-harvest processing, transportation, and storage. Further, contamination by microbial and chemical agents (pesticides, herbicides, heavy metals), as well as infection by pest and pathogens can alter the chemical constituents of the plants. Qualitative analysis of three important secondary metabolites viz. Phenolics, Flavonoids and Tannins showed that *Centella asiatica* has maximum contents of Flavonoids and Phenolics. *Lawsonia inermis* showed maximum contents of Tannin. These results are in favour of the various reports on the broader and more effective use of *Centella asiatica* in various health problems, the property of which are depicted here in the present qualitative analysis.

CONCLUSION

Phytochemical study is crucial for assessing a plant's potential medicinal uses as well as identifying the active principle underlying the plant's recognized biological activities. Additionally, it offers the foundation for more accurate research and focused molecule isolation. The type of solvent used has a major impact on the phytochemical's ability to be extracted from plant material. To ascertain if a phytochemical is present in the sample or not, the same test was used for phytochemical analysis. Therefore, for more accurate results, two or more separate tests should be run. All things considered,

the results validate the ethnomedical value of *C. asiatica* and *L. inermis* and offer a solid scientific foundation for further research on them. The study is a first step toward quantitative analysis, bioactive chemical isolation, and assessment of the compounds' pharmacological and antibacterial properties.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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