Qualitative phytochemical assessment of *Leucas aspera* (Willd.) Link using various solvent extracts

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**Article History:**
- Received on: 01 Aug 2020
- Revised on: 01 Sep 2020
- Accepted on: 07 Sep 2020

**Keywords:**
Flavonoids, Leucas Aspera, Methanol extract, Phytochemical, Phenols, Secondary metabolites

**ABSTRACT**

*Leucas aspera* (Willd.) Linn. belonging to family Lamiaceae is well-known as 'Thumbai' in Tamil Nadu with traditional medicinal value as an antipyretic and insecticide. The current research work has been carried out to screen the phytochemical content of the aqueous, hexane, ethanol and methanol extract from the whole plant of *Leucas aspera*. The percentage of yield of the extracts varied according to the organic solvents used in which aqueous yielded 5.4% and methanol yielded 7.8%, respectively. Among the 11 phytochemicals screened, the whole-plant extract showed the presence of 10 phytochemicals. The phytochemical screening result showed that the carbohydrate, protein, lipids, alkaloids, saponins, glycosides, tannins, flavonoids, triterpenoids and phenols were present in the plant sample. Among the different solvent extract, methanol extract exhibited more number of phytochemical presence, and aqueous extract showed the least number of phytochemical presence. Among the different phytochemicals screened, carbohydrates, alkaloids, saponins, glycosides, tannins were present in all the solvent extracts. In contrast, steroids were absent in all the solvent extracts, and lipid was present only in methanol extract. The result suggested that the presence of secondary metabolites of *Leucas aspera* could be a potential source for antimicrobial, antioxidant activity and cytotoxic activity and the methanolic extract of *Leucas aspera* could be explored for its potent pharmacological activities.

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ISSN: 0975-7538
DOI: [https://doi.org/10.26452/ijrps.v11iSPL4.4224](https://doi.org/10.26452/ijrps.v11iSPL4.4224)

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**INTRODUCTION**

Nature has provided medicines via useful phytochemicals present in plants from time immemorial. Thousands of years, phytochemicals are having been an essential source of medicine. Plants are a vital ingredient of the primary health-care system as a traditional medicine in many countries, namely India, Pakistan, Sri Lanka, China, Bangladesh, etc. (Karunamoorthi et al., 2013). In daily life, even nowadays, numerous medicinal plants like herbs and shrubs have been used to combat diseases. The consumption of vegetables, fruits, leaves and herbs have been linked with a reduction of severity of diseases such as cancer (Kumar et al., 2013). Over the past ten decades, the development of chemotherapy and technology for the production of synthetic drugs changed the world scenario in medicine (Sofowora et al., 2013). The extensive application of commercial drugs which are synthetic and non-nature lead to side effects and mainly, development of drug resistance in the majority of the pathogenic microbes is a most critical problem (Fair and Tor, 2014). To minimize the synthetic drugs and to reduce side effects, in recent decades, researchers have an interest in the
investigation on the usage of plants by screening, and the examination on phytochemicals, metabolite and biological activities from plants has been expanded. Bioactive phytochemicals from plants currently receive more awareness because of their non-side effects (Anand et al., 2019). Considering the awareness of side effects, it is important to screen the chemical content of plants to assess their potential pharmacological activities directly or indirectly. *Leucas aspera* (Willd.) Linn. belonging to family Lamiaceae is well-known as ‘Thumbai’ in Tamil Nadu, which is found all over India. The plant has traditional medicinal value as an antipyretic and insecticide. Locally, the fresh leaves are applied against snake bites (Shirazi, 1947). The plant is also indicated in traditional medicine for cough, cold, painful swelling and chronic skin eruption (Chew et al., 2012). Hence, the objective of the current research work was to screen the phytochemical content of the *Leucas aspera* whole plant using aqueous, hexane, ethanol and methanol extract.

**MATERIALS AND METHODS**

**Plant material**

The whole fresh plants of *Leucas aspera* (Wild.) Linn., collected randomly from Vellore district of Tamil Nadu, India, were washed in running tap water to get rid of dust and other epiphytic organisms. After washing, the plant was shade dried for a week and was finely powdered using a mixer grinder. The ground powder was stored in sterile bottles in the dark until further use.

**Preparation of extracts**

The powdered samples were extracted with various solvents such as aqueous, hexane, ethanol, and methanol by cold extraction method. The extraction was carried out as 1:10 (v/v), i.e. 100 ml of solvent was used to soak 10 gm of powdered plant samples in a conical flask. The soaked flask was periodically shaken. After five days, the extract containing phytochemicals were filtered using Whatman filter paper and stored until further studies.

**Phytochemical screening**

Various solvent extracts of *Leucas aspera* were screened for phytochemical constituents according to standard methods (Harborne, 1998).

**Carbohydrates**

*Malisch’s test*

In a test tube having 2ml of the extract, 1ml of α-naphthol solution followed by conc. Sulphuric acid was added carefully. The reddish-violet colour at the intersection of the two fluids indicates the existence of carbohydrates.

*Benedict’s test*

In a test tube having 2ml of the extract, 5 ml of Benedict’s reagent was added and boiled for 2 min. After cooling, red precipitate creation indicates the existence of sugars.

*Fehling’s test*

A test tube, having 1ml of the extract along with equal quantities of Fehling solution A and B, upon heating creation of a red brick precipitate indicates the existence of reducing sugars.

**Proteins**

*Biuret Test*

In a test tube having 1 ml of the extract, 4% NaOH and few drops of CuSO4 solution were added and treated. Creation of purple violet colour indicates the existence of the presence of proteins.

*Ninhydrin Test*

In a test tube having 1 ml of the extract, three drops of 5% Ninhydrin solution was added and treated in boiling water bath for 10 mins. Creation of purplish or bluish colour indicates the existence of proteins, peptides or amino acid.

*Xanthoproteic test*

A test tube having 1 ml of the extract was treated with 1 ml of concentrated nitric acid. The test tube was boiled after a white precipitate formation. After cooling, of ammonia or 20%, sodium hydroxide is added. Creation of orange colour indicates the existence of amino acids.

*Millon’s Test*

In a test tube having 1 ml of the extract, 1 ml Millon’s reagent was added and mixed well. Creation of whitish-yellow coloured hasty indicates the existence of alkaloids.

**Lipids**

*Saponification Test*

In a test tube having 1 ml of the extract, an equal amount of alcoholic potassium hydroxide was added and boiled on a water bath. Creation of soap indicates the existence of lipids.

**Alkaloids**

*Mayer’s test*

In a test tube having 1 ml of the extract, 1 ml Mayer’s reagent was added and mixed well. Creation of whitish-yellow coloured hasty indicates the existence of alkaloids.
Table 1: Yield percentage of various solvent extracts of *Leucas aspera*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Solvent</th>
<th>Yield (% W/W)</th>
<th>Extract colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aqueous</td>
<td>5.4</td>
<td>Dark green</td>
</tr>
<tr>
<td>2</td>
<td>Hexane</td>
<td>5.9</td>
<td>Dark green</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>6.4</td>
<td>Dark brown</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>7.8</td>
<td>Dark greenish-red</td>
</tr>
</tbody>
</table>

**Hager’s Reagent**

In a test tube having 1 ml of the extract, 3 ml of Hager’s reagent was added. Creation of yellow coloured hasty indicates the existence of alkaloids.

**Wagner’s test**

In a test tube having 1 ml of the extract, 2 ml of Wagner’s reagent was added and mixed well. Creation of reddish-brown precipitate indicates the existence of alkaloids.

**Dragendorff’s test**

In a test tube having 1 ml of the extract, an equal amount of dragendorff’s reagent was added and mixed well. Creation of an orange-red hasty indicates the existence of alkaloids.

**Saponins**

**Foam test**

In a test tube, around 20 ml of distilled water was added with a little amount of extract for dilution. After dilution in a graduated cylinder, it was shaken for 15 min lengthwise. Development of foam for 1 cm layer indicates the existence of saponins.

**Lead acetate test**

In a test tube having 1 ml of sample, 1% lead acetate solution was added and boiled. Creation of white hasty indicates the existence of saponins.

**Glycosides**

**Keller-Kiliani test**

In a test tube having 0.5 ml of the extract, equal amount of water and conc. A solution of lead acetate was added and filtered. The newly formed filtrate is taken in a china dish, and 2 ml of chloroform was added, shaken and evaporated. After evaporation, 3 ml of glacial acetic along with few droplets of ferric chloride to deliquesce the remaining filtrate and finally 2 ml of conc. Sulphuric acid was added. Formation of reddish-brown gradually turns to bluish green indicates the existence of glycosides.

**Legal’s Test**

In a test tube having solvent extract, few ml of pyridine along with few drops of nitroprusside and two drops of sodium hydroxide was added and mixed. Intense red colour indicates the existence of glycosides.

**Borntrager’s test**

In a test tube having 1 ml of the solvent extract, 5 ml of dilute hydrochloric acid was added, boiled and filtered. The filtrate was mixed with benzene or chloroform along equal with the amount of ammonia and shaken well. The creation of pink to red colour indicates the existence of anthraquinone glycosides.

**Tannins**

**Gelatin Test**

In a test tube having 1 ml of solvent extract, 1% aqueous solution of gelatin and 10% sodium chloride were added. The creation of white coloured hasty indicates the existence of tannins.

**Ferric chloride test**

In a test tube having 1 ml of extract, 1ml of 10% ferric chloride solution was added. The creation of dark blue indicates the existence of gallic tannins, and the greenish-black indicates the existence of catechol tannins.

**Lead Acetate Test**

In a test tube having 1 ml of extract, 3 ml of 10% basic lead acetate solution was added—the creation of white coloured hasty shows the existence of tannins.

**Flavonoids**

**Alkaline Reagent Test**

In a test tube having 2 ml of extract, few drops of 2N sodium hydroxide were treated. The formation of deep yellow colour shows the existence of flavonoids.

**Shinoda Test**

In a test tube having 1 ml of extract, two to three pieces of magnesium chips were added, followed by a few drops of conc. HCL. A pink to red colouration shows the existence of flavonoids.

**Steroids**

**Saikowski’s test**

In a test tube having 1 ml of extract, 2 ml conc. $\text{H}_2\text{SO}_4$ was added. Red colouration indicates the existence...
Table 2: Qualitative phytochemical analysis of various solvent extracts of *Leucas aspera*

<table>
<thead>
<tr>
<th>phytochemical test</th>
<th>Aqueous</th>
<th>Hexane</th>
<th>Ethanol</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molisch’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Benedict’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fehling’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Biuret Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ninhydrin Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Xanthoproteic Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Millon’s Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saponification Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayer’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hager’s Reagent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Wagner’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dragendorff’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foam test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lead acetate test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keller-Kiliani test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Legal’s Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Borntrager’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lead Acetate Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous NaOH</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaline Reagent Test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Shinoda Test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Conc. H2SO4</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mg-HCl</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lead Acetate test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salkowski’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Libermann’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeCl3 solution test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lead acetate test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
of steroids.

**Libermann’s test**

In a test tube having 1 ml of extract, few ml of acetic acid solution was added after ice-cooled conc. H$_2$SO$_4$ was added carefully. The bluish-green colouration shows the existence of steroids.

**Triterpenoids**

**Triterpenoids Test**

In a test tube having 2 ml of extract, 1 ml of chloroform was added and evaporated followed by treatment with conc. H$_2$SO$_4$. A grey or deep brown colouration shows the existence of terpenoids.

**Phenols**

**FeCl$_3$ Test**

In a test tube having 2 ml of extract, five drops of 5% FeCl$_3$ solution was added and stirred well. A bluish brown colouration shows the existence of phenol.

**Lead acetate test**

In a test tube having 3 ml of extract, 3 ml of 10% lead acetate was mixed. The precipitation appearance shows the existence of phenol.

**RESULTS**

Various organic solvents namely aqueous, hexane, ethanol and methanol were used to extract the phytochemicals from the whole plant of *Leucas aspera* (Willd) Link. The percentage of yield of the extracts varied according to the organic solvents used. The yield of different solvents extract was expressed in Table 1. Among the four different solvents, methanol yielded maximum extract of 7.8%, which is followed by ethanol, whereas aqueous yielded minimum extract of 5.4%. The colour of the extract was found to be deep green in aqueous and hexane extract, dark brown in ethanol extract and dark greenish-red in methanol. In this study, the qualitative analyses of phytochemicals for the four extracts have been done and found a broad range of phytochemical compounds which is shown in Table 2. Phytochemical screening of *L. aspera* suggests that both primary and secondary metabolites were present in the whole plant (Table 2). The yield, as well as the presence of phytochemicals, was maximum in the methanol extract of *L. Aspera*. The hexane being highly nonpolar, was able to extract significantly less compound compared to other solvents. Among the four different solvents, methanol was found to contain all the phytochemicals tested except steroids which are absent in all the solvent extract. Carbohydrates, alkaloids, saponins, glycosides, and tannins were present in all the solvent extracts of *Laspera*. Steroids were absent in all the solvent extracts. Lipid was found only in the methanol extract and absent in aqueous, hexane and ethanol extracts.

**DISCUSSION**

The local awareness of medicinal plants was as oral information (Majumdar and Datta, 2007; Debarma et al., 2017). To realize the beneficial process of medicinal plants, it is essential to screen the phytochemicals as a first step. At first, qualitative analysis of phytochemicals was carried out with various reagents or chemical substances to identify the presence in each extract. From the study, it is clear that the polarity of solvents determines the extract yield except aqueous. In the present study, the *L. aspera* revealed the presence of 10 phytochemicals out of 11 phytochemicals screening of various solvent extracts. Triterpenoids were present in this plant which is ubiquitous among the plant kingdom, and several triterpenoids possess anticancer and cytotoxicity property against several tumour cell line as well as in preclinical animal models along with anti-inflammatory and anti-proliferative (Patlolla and Rao, 2012; Bishayee, 2011). (Anantharaju et al., 2016) stated plant-based phenolic molecules had been known to exhibit anticancer mechanism by altering genes controlling crucial processes such as initiation and progression of cancers. Because of the above statement, the presence of phenols in this experimental plant shows that this plant can be used to inhibit cancer. Currently, flavonoids are considered as a vital constituent in multiple sectors such as medicinal, pharmaceutical, nutraceutical and cosmetic applications are given their properties such as anti-inflammatory, antimutagen, anticarcinogen and antioxidant combined with their ability to regulate crucial enzymatic function (Panche et al., 2016; Kumar and Pandey, 2013). Saponins are naturally occurring substances widely found in plant cells. They are identified by their property to form soap-like foam formation in aqueous solution. Saponins have excellent medicinal values. They are found to reduce blood cholesterol and acts against cancer by inhibiting the growth of tumours and by various other key enzyme pathways (Man et al., 2010).

**CONCLUSIONS**

From the present study, it may be concluded that the phytochemicals of *L. aspera* can be used in different food and pharmaceutical fields. Since this plant contains most of the secondary metabolites which are liable for various biological actions; this study would be providing information for selection of the extract...
for biological activity and isolation of phytochemicals responsible for the activity.

ACKNOWLEDGEMENT

The authors are thankful to the Head, Department of Botany, Annamalai University, for providing the laboratory facilities.

Conflicts of interest

The author(s) declare that they have no conflicts of interest concerning the research work, authorship and publication of this article.

Funding support

The authors declare that they have no funding support for this study.

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