Preliminary phytochemical screening and antioxidant activity of *Emilia sonchifolia* (L.) DC., a member of ‘Dashapushpa’

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Received: September 03, 2018  Accepted: October 28, 2018

**ABSTRACT**  *Emilia sonchifolia* belongs to the family Asteraceae. It is known as Muyalcheviyan in Malayalam. The plant is 30-40cm in height and glabrous slender herb. Leaves are obovate and flowers are purplish in colour. It is also known as Cupid’s shaving brush. The plant is usually found in waste grounds and moist areas. The aerial part of the plant has been reported to contain alkaloids, flavonoids, and terpenes. The whole plant is used for various treatments. Therefore, the main objectives of the present study are screening of various phytochemicals and antioxidant activity of methanolic whole plant extract of *E. sonchifolia*. Phytochemical screening showed the presence of alkaloids, terpenoids, carotenoids, flavonoids and tannins. Antioxidant activity of the methanolic extract of was *E. sonchifolia* 65.28% for 1mg/ml. The results obtained in this study confirms antioxidant potential of *E. sonchifolia*.

**Keywords:** *Emilia sonchifolia*, DPPH, Spectrophotometric assay.

**INTRODUCTION**

Plants have been the major sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements and cosmetics in the recent days. India is well known for its plant diversity and is rich in medicinal plant wealth. It hosts three biodiversity hotspots: the Western Ghats, the Eastern Himalayas, and the hilly range that straddle the India-Myanmar border. These hotspots have numerous endemic species. Kerala is one of the lovely states in India famous for scenic beaches and serene backwaters. The Western Ghats of Kerala is famous for its medicinal plant wealth and the tradition of indigenous system of therapy specifically the Ayurveda.

In India, the Ayurvedic system of medicine has been in use for over three thousand years. Charaka and Susruta, two of the earliest Indian authors had sufficient knowledge of the properties of the Indian medicinal plants. The medicinal form is governed by the laws of nature, which suggest that life is a combination of senses, mind, body and soul. This holistic approach gained worldwide acceptance to Ayurvedic treatments. Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural products, organic chemistry and plant biochemistry. It deals with the enormous varieties of organic substance that are synthesised and accumulated by plants and deals with the chemical structures of these compounds, their biosynthesis, turnover and metabolism, their natural distribution and their biological function. Challenge of phytochemistry is to carry out these methods which are needed for separation, purification and identification of many different constituents present in plants through operations on small amount of material. Phytochemical process has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals and through chromatographic techniques. It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others. A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems. The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defence mechanism. Antioxidants are those substances which posses free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury.

*Emilia sonchifolia* belongs to the family Asteraceae. It is known as Muyalcheviyan in Malayalam. The plant is 30-40cm in height and glabrous slender herb. Leaves are obovate and flowers are purplish in colour. It is also known as Cupid’s shaving brush. The plant is usually found in waste grounds and moist areas. The aerial part of the plant has been reported to contain alkaloids, flavonoids, and terpenes. The whole plant is used for various treatments. A tea made from the leaves is used in the treatment of dysentery. The leaf juice
is used in treating eye inflammations, night blindness, wounds and sore ears. Root is used as antidiarrheal. Fresh juice and methanoic extract of *E. sonchifolia* leaves reported to possess anti-inflammatory and antioxidant activities. The water extract of this plant showed antimicrobial activity (Arun Raj *et al.*, 2013).

The present study was designated to investigate the preliminary phytochemical analysis and evaluation of antioxidant activity of the methanolic extract of the whole plant of *Emilia sonchifolia*.

**MATERIALS AND METHODS**

The plant was collected from naturally growing population of *Emilia sonchifolia* Kerala University Campus, Karivattom, Thiruvananthapuram. Phytochemical test were carried out using standard procedure to identify constituents. The plant was properly identified with the help of authentic literature and documented with their characteristic features and a voucher specimen has deposited in the Department herbaria (KUBH-10121).

**Preparation of plant extract**

The entire plant was used for the assay. The plant materials were washed and shade dried and chopped into small pieces for grinding. The plant material was powdered in an electric mixer. The powdered plant material was kept in air tight container with proper labeling for future use. The plant powder was extracted in a single solvent methanol. 10g of plant powder was extracted in 200ml of methanol for about 4-5 hours in Sohlet apparatus at room temperature. The extract was collected and evaporated in an oven at a temperature of 55°C. It was collected in a Petri dish, weighed and was stored in cold condition for further studies. The dried extract thus obtained was used for the phytochemical analysis, analysis of antimicrobial and antioxidant property using various standard procedures.

**Qualitative preliminary phytochemical screening**

**Phytochemical screening**

Chemical test were carried out using standard procedure by Harborne, J. B. 1998 to identify the constituents present in the plant.

**Detection of alkaloids**

Solvent free extract (50mg) was mixed with few drops of dil.HCl and was then filtered. Test for alkaloids was carried out in this filtrate.

**Test 1- Mayer's Test**: One or two drops of Mayers reagent (mercuric chloride 1.36g dissolved on 60ml distilled water and mixed in a solution of 5g of potassium iodide in 10ml distilled water) was added to the filtrate through the side of the test tube, formation of white creamy precipitate indicates the presence of alkaloids.

**Test 2- Dragendorff's Test**: The reagent (0.85g bismuth nitrate dissolved in 40ml distilled water and 10ml glacial acetic acid, followed by addition of 5g potassium iodide dissolved in 10ml distilled water) was added to the filtrate. Formation of prominent yellow precipitate indicates the presence of alkaloids.

**Test 3- Wagner's Test**: Reagent (1.27g of iodine and 2g potassium iodide dissolved in 5ml distilled H₂O) was added to the filtrate. Formation of reddish brown precipitate indicates the presence of alkaloids.

**Detection of glycosides**

**Molisch test**: Two ml of the prepared filtrate were mixed with 0.2 ml of alcoholic solution of α-naphthol 10% and 2 ml of sulphuric acid, a reddish violet zone is formed, this indicates the presence of carbohydrates or glycosides.
Detection of terpenoids
Salkowski test: Five ml of extract was mixed with 2ml of chloroform and about 3ml of con.H₂SO₄ was carefully added. At the separation level of the two liquids, a reddish-brown ring forms, which indicates the presence of terpenoids.

Detection of carotenoids
About 0.02g of plant extract was mixed with chloroform, mixed well and then the mixture was filtered. To the filtrate, conc.H₂SO₄ was added, formation of a blue colour at the interface indicate the presence of carotenoids.

Detection of steroids
Libermann-Burchard test: One ml of extract was treated with 0.5ml of acetic anhydride and 1ml of H₂SO₄ carefully. A colour change from violet to blue or green indicates the presence of steroids.

Detection of saponin
Foam test: About 0.5gm of extract was mixed with 2ml of distilled water and heated for few minutes and filtered. The filtrate was vigorously shaken. The persistent froth was observed for 10 minutes, this indicates the presence of saponins.

Detection of flavanoids
The extract was shaken with 1ml of dilute ammonia solution and con. H₂SO₄. Formation of yellow colour indicates the presence of flavanoids.

Detection of phenol
To the plant extract, a few drops of 1% aqueous or alcoholic ferric chloride was added. The formation of bluish-black colour indicates the presence of phenol.

Detection of quinine
One ml of the plant extract was mixed with 5ml of con. HCl. The formation of yellow precipitate indicates the presence of quinone.

Detection of tannin
The sample was mixed with distilled water and boiled for 5 minutes and was filtered and was used for the test.
Two drops of 10% ferric chloride was added to 1ml of the filtrate. Formation of bluish or greenish or brownish black colour indicates the presence of tannins.

Spectrophotometric assay for the evaluation of antioxidant activity of methanolic plant extract using DPPH
Free radical scavenging activity by DPPH and spectrophotometric assay
DPPH radical scavenging activity of methanolic extract of the plants were tested for the antioxidant activity. The H-donor activity of the extract was estimated in this method. Different concentrations of methanol extract ranging from 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1mg/ml was prepared. Hundred ml of DPPH radical solution in methanol was freshly prepared. One ml extract of different concentrations was added to 2ml of DPPH solution and the reaction mixture was incubated at 37ºC for 20 minute. The absorbance was read at 517nm against positive control which do not contain the extract. The assay was carried out in triplicate. A decrease in absorbance of DPPH solution indicates an increase in DPPH scavenging activity. The activity is given as percentage DPPH radical scavenging.

% inhibition= \( \frac{(A_b - A_s)}{A_b} \times 100 \)

Ab- absorbance of control
As- absorbance of sample

RESULTS
Phytochemical evaluation
Preliminary phytochemical analysis of methanolic extracts of the plants was done by using various preliminary analysis tests. Preliminary phytochemical analysis is done to identify the major groups of phytochemical present in the plant samples. Preliminary analysis results are shown in the table (Table.1).

Table 1 Preliminary screening of methanolic extract of whole plant of E. sonchifolia

<table>
<thead>
<tr>
<th></th>
<th>Methanolic extract of E. Sonchifolia</th>
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<tbody>
<tr>
<td>Alkaloid</td>
<td>+++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>++</td>
</tr>
<tr>
<td>Carotenoid</td>
<td>++</td>
</tr>
<tr>
<td>Steroid</td>
<td>-</td>
</tr>
</tbody>
</table>
‘+’ sign indicate presence and ‘-’ sign indicate absence. Number of ‘+’ sign indicate the intensity.

The methanolic extract of the plant showed the presence of different phytochemicals like alkaloids, terpernoids, carotenoids, flavanoids and tannin.

**Antioxidant activity**

**Free radical scavenging activity by DDPH spectrophotometric assay**

The DDPH assay was used to measure the antioxidant activity of the plant extract as it offers a rapid technique to screen the antioxidant property. The antioxidant values (percentage of inhibition) of the crude methanolic extract were examined.

The percentage of scavenging activity of DPPH radical was found to be concentration dependent i.e. concentration of the extract between 0.2 - 1mg/ml increasing the inhibition activity. Methanolic extract of whole plant show 14.53%, 29.98%, 44.29%, 54.21% and 65.28% of inhibition in 0.2, 0.4, 0.6, 0.8 and 1mg/ml concentration of extract respectively. From the result it is clear that highest scavenging was 65.28% at 1mg/ml concentration (Table.2).

**Table 2. Antioxidant property of methanolic extract of whole plant of E. sonchifolia**

<table>
<thead>
<tr>
<th>Conc. of sample (mg/ml)</th>
<th>DPPH</th>
<th>Sample</th>
<th>Absorbance at 517 nm</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2ml</td>
<td>1ml methanol</td>
<td>0.867</td>
<td>0</td>
</tr>
<tr>
<td>Blank</td>
<td></td>
<td>3ml methanol</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.2</td>
<td></td>
<td>1ml sample</td>
<td>0.741</td>
<td>14.53</td>
</tr>
<tr>
<td>0.4</td>
<td></td>
<td></td>
<td>0.607</td>
<td>29.98</td>
</tr>
<tr>
<td>0.6</td>
<td></td>
<td></td>
<td>0.483</td>
<td>44.29</td>
</tr>
<tr>
<td>0.8</td>
<td></td>
<td></td>
<td>0.397</td>
<td>54.21</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>0.301</td>
<td>65.28</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Herbal drugs play an important role in healthcare programme especially in developing countries. There is a need for documentation of research work carried out on traditional medicine and also it becomes extremely important to make an effort towards standardisation of plant materials to be used as medicine. Secondary metabolites are molecules that are not necessary for the growth and reproduction of plants, but may serve some role in plant defence mechanism. They act as phytoalexins, killing bacteria that the plant recognizes on a threat. Successive isolates of botanical compound from plant material is largely depending on the type of solvent used in the extraction procedure. In the present study, solvent system used extraction was methanol. In Soxhlet extraction, the sample is continually exposed to fresh solvent that can withstand the temperature of the boiling solvent. The traditional healers use water as the solvent but later it was clear that the plant extract by methanol provide more compounds (Jigna and Sumitra, 2007).

**Phytochemical evaluation**

**Qualitative preliminary phytochemical screening**

Phytochemical analysis revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Preliminary analysis is much helpful for the screening of secondary metabolites and other biochemical present in the plant.

Alkaloids and flavanoids have been linked or suggested to be involved with antibacterial and antiviral activity while tannis and flavanoids are thought to be responsible for antidiarrheal activity (Enzo, 2007). This could explain the role of the plant material as an antimicrobial agent (Pithayanukul et al., 2007). The phytochemical analysis of some of the plants belonging to the group of *Dashapushpa* was reported (Deepan et al., 2012; Majumder et al., 2012).
The result of the present study revealed that the plant material used for preliminary phytochemical screening possessed alkaloids, terpenoids, carotenoids, saponin and flavanoids (Mamta Raj, 2012; Singh and Bhat, 2003) reported that flavonoids are responsible for the antimicrobial activity associated with some ethnomedicinal plants. According to the results obtained in this study it is suggested that the identified phytochemical compounds may be the bioactive constituents

**Antioxidant evaluation**

Several studies have been carried out to study the antioxidant properties possessed by different plants. Different classes of phytochemicals and several plant extracts have been found to have quite prominent antioxidant activity (Tripathi et al., 1996; Rao, 1997; Vani et al., 1997).

Flavanoids are groups of naturally occurring compounds widely distributed, as secondary metabolites in the plant kingdom. These flavanoids have also been reported to possess antioxidant and antiradical properties (Nakayoma and Yamada, 1995).

DPPH is one of the free radical widely used for testing preliminary radical scavenging activity of a compound or a plant extract. The DDPH test (Wagner, 1996) provides information as the reactivity of test compounds with stable free radical. Because of its odd electron, 2, 2- diphenyl 1- picryl hydrazyl radical (DPPH) gives a strong absorption band at 517nm (Duh et al., 1999). DPPH radical is scavened by antioxidants through the donation of a proton forming the reduced DPPH. The colour changes from purple to yellow after reduction which can be quantified by its decrease of absorbance.

As antioxidants have been reported to prevent the oxidative damage caused by the free radical, it can interfere with the extraction process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers. Phenolic compound and flavanoids are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc (Miller, 1996).

The antioxidant activities of the methanolic extract of the plants were carried out using DPPH (2, 2-diphenyl 1- picryl hydrazyl). The plant showed antioxidant activity was below 50%. The phenolic compounds may contribute directly to antioxidant action (Shylesh, B. S., Padikkala, J. 2000; Duh et al., 1999). Hence the low antioxidant property may be due to the lack of phenolic compound.

**CONCLUSION**

Medicinal plants are now being used as model for antimicrobial agents and it is believed that plant based drug cause less or no side effects when compared with synthetic antibiotics. Phytochemical analysis helps to identify the presence of major phytochemicals like alkaloids, terpenoids, carotenoids, flavanoids and tannin. The antioxidant study help to analyze the activity of the plant and helps in the development of new drugs for the treatment of various diseases. The results obtained from this study confirm antioxidant activity of *Emilia sonchifolia*.

**ACKNOWLEDGEMENT**

The authors thank, the Head, Department of Botany, University of Kerala for encouragement and providing facilities for the completion of work.

**REFERENCES**


