

## ***In vitro* Studies on Singapore Daisy (*Sphagneticola trilobata*) and formulation of dip drink**

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### **To Cite this Article**

Caroline Arunagiri, Ann mariya stanly, Dharini Shanmugham, Srilakshmi Arunachalam, Yaashvi Vijay Bharath "***In vitro* Studies on Singapore Daisy (*Sphagneticola trilobata*) and formulation of dip drink**", *Journal of Science and Technology*, Vol. 07, Issue 02, Mar-April 2022.

### **Article Info**

Received: 26-04-2022

Revised: 18-04-2022

Accepted: 26-04-2022

Published: 30-04-2022

**Abstract-** The leaves of *Sphagneticola trilobata*, a plant belonging to the Asteraceae family, are rich in anti-oxidants and nutrients. The leaf extract shows no sign of toxicity at levels less than 2500 mg and is considered as safe to consume. The phytochemical screening of compounds such as alkaloids, flavonoids, polyphenols, carbohydrates and tannins is studied. The leaves are analysed for its drying characteristics using Hot Air Oven at 163°C and is packed in Tea Filter Bags. The free radical scavenging activity is studied in-vitro by measuring DPPH. Antimicrobial activity against four microorganisms, Staphylococcus aureus, E.Coli, Yeast and mould and Salmonella, using agar well diffusion method was evaluated. Biochemical tests to determine the amount of alkaloids, flavonoids, phenolic compounds and carbohydrates on the dried leaves were examined. The formulated product, Daisy Tea was analysed based on pH and acidity, bulk density, moisture content and ash content.

**Keywords**— *Sphagneticola trilobata*, Phytochemical analysis, DPPH assay, Microbial activity, Dip Drink.

## **I. INTRODUCTION**

*Sphagneticola trilobata*(L.)Pruski, commonly known as trailing daisy, creeping-oxeye, *wedelia*, Singapore daisy belongs to the *Asteraceae* family and is cultivated for ornamental purposes. It is known as an invasive weed and grows along roadsides, agricultural lands, canals and streams. The leaves of *Sphagneticola trilobata* is known for its medicinal properties and is used as an ancient traditional medicine in various countries. One of the plants that consist of therapeutic effects that are enriched with antioxidant property is *S.trilobata*. Numerous phytochemical components like alkaloids, saponins, tannins, phenol, terpenoids and flavonoids are present in the leaves of *S.trilobata*. Works of literature confirmed that *S.trilobata* could be a treatment for various diseases like backache, muscle cramp, diabetes, rheumatism and arthritic painful joints. It is generally considered a safe herb without affecting hepatic and renal functions.

Anti-oxidant has strong control over free radicals that causes deterioration in human tissues as it belongs to ROS. This dip drink provides anti-oxidants that helps us fight against the free radicals present in our body. The accumulation of free radicals can be controlled by the intake of anti-oxidant rich foods. Subsequently, the aqueous extract of *S.trilobata* leaves showed active resistance against bacterial growth but did not have any resistance against fungal growth. Works of Literature also confessed that the extract of the plant has hepato-protective activity against liver injuries.

Dried leaves of *S.trilobata* are packed in the dip bag and the diffusion rate is a considered as it decides the essence of the extract in hot water. Daisy tea's medicinal properties using the phytochemical components and anti-oxidant value proves to be a highly beneficial drink when instant medical attention is needed. Dip bags depend on parameters such as material used, pore size, shape, loading capacity, diffusion rate, holding time and temperature. A product considering the above-mentioned attributes is prepared and is named as DAISY TEA.



**Fig 1.1:** *Sphagneticola trilobata* L. Pruski, Avinashilingham institute campus, Coimbatore.

## II. MATERIALS AND METHODS

### 1. Collection and Preparation of *S.trilobata* leaves extract:

The healthy leaves of *S.trilobata* are collected from Avinashilingham institute campus. They are washed with clean water to remove insects and impurities. With the help of Mortar and Pestle, the leaves are crushed consistently until the aqueous extract is removed.

### 2. Phytochemical screening of *S.trilobata* aqueous extract:

#### **Test for flavonoids:**

Appearance of yellow colouration when dilute ammonia solution is added to the aqueous extract with a few drops of conc.H<sub>2</sub>SO<sub>4</sub> indicates the Presence of **flavonoids**.

#### **Test for alkaloids:**

When Mayer's and Dragendroff's reagent is added to the extract, a precipitate occurs which proves the presence of **alkaloids**.

#### **Test for Phenolic compounds:**

**Phenol compounds** is detected when FeCl<sub>3</sub> and ethanol is added to 2 ml aqueous extract.

#### **Test for Carbohydrates:**

Drops of molisch's reagent were added to the extract and the con.H<sub>2</sub>SO<sub>4</sub> on the sides of the test tube and formation of a ring shows the presence of **Carbohydrates**.

**Test for cholesterol:**

Addition of chloroform and acetic acid to the aqueous extract of *S.trilobata* indicates the Presence of **Cholesterol** levels.

**Test for tannins:**

Appearance of yellow precipitate when few drops of 1% lead acetate added to 5 ml of plant extract showed the presence of **tannins**.

**Test for steroids:**

The colour change from violet to blue when acetic anhydride is added to the sample indicated the presence of **steroids**.

**Test for saponins:**

The formation of foam when the extract was agitated in a graduated cylinder showed the amount of **saponins** present.

**Test for anthocyannins:**

When 2 ml of NH<sub>4</sub>Cl and ammonia is added to the aqueous solution of *S.trilobata*, the pink-red changes to blue-violet, indicating the presence of **anthocyanins**.

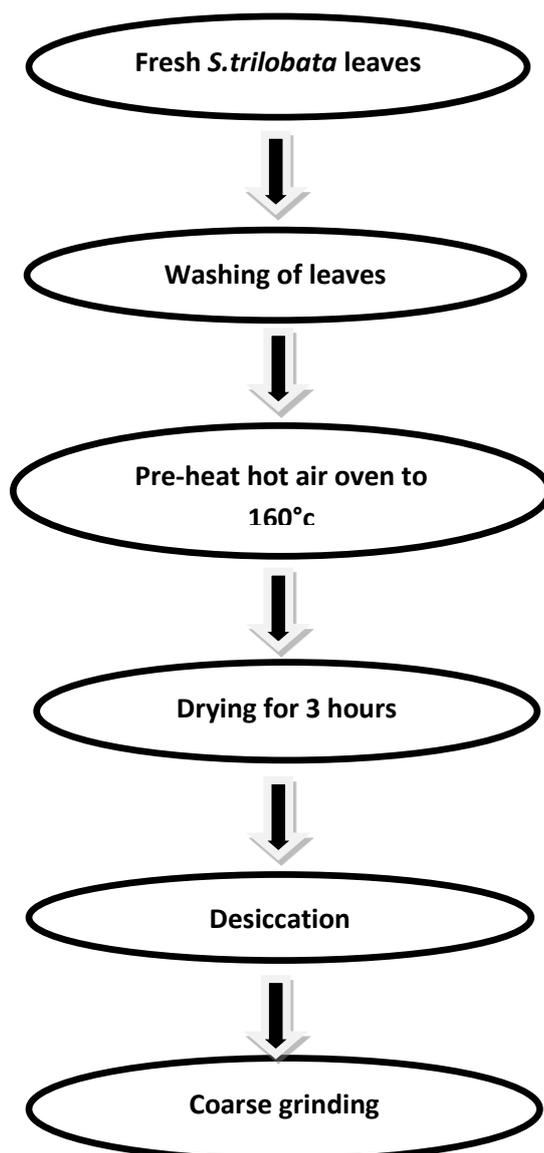
**Test for quinones:**

Conc.HCl added to 2 ml of extract gives a green colour showing the presence of **quinones**.

**3. Formulation of Dip Drink:**

The dried leaves are then cooled by keeping it in a desiccator for 10 minutes. Thereafter, the dried leaves are grinded coarsely.

According to Areeya Suchantabud et.al, rats received *S.trilobata* extract at 1000, 1500 and 2000 mg/kg. No signs of toxicity were found at these levels. Daisy tea dip bag was filled with 1000 mg (1 g) of dried leaves based on consumer acceptability and other products' net weight <sup>[22]</sup>.





**Fig.2.1:** Preparation of Daisy Tea Dip drink

**4. Moisture content:**

50 g of dried *S. trilobata* leaves are weighed accurately in a previously dried and tared dish. The dish is placed in the oven for 3 hours with its lid underneath. The moment the oven attains 103°C after the dishes are placed, the time is reckoned. The dishes are weighed after removing according to the time limit of 3 hours and are cooled down in desiccators. The dish should be placed back in the oven at half hour intervals till constant weight is achieved and the moisture content is calculated.

**5. Ash content:**

The coarse *S.trilobata* leaves are weighed in a crucible. The muffle furnace is preheated to 600°C. The crucible is placed in the muffle furnace. After three hours, the crucible with ash is weighed again and ash content is calculated.

**6. pH and acidity:**

The pH may be determined by measuring the electrode potential between glass and reference electrodes in the dip drink and the pH meter is standardized using standard pH Buffers. To determine acidity, the fat is extracted and weighed about 5 to 10 g of extracted fat in a 250ml conical flask 50ml to 100ml of freshly neutralized hot ethyl alcohol and about 1ml of phenolphthalein indicator solution is added. The mixture is boiled for about 5 minutes and titrated while hot against standard alkali solution shaking vigorously during titration. The weight of the oil taken for estimation and the strength of the alkali used for titration shall be such that volume of alkali required for titration does not exceed 10ml.

**7. Bulk density:**

The bulk density for the dip drink is measured by weighing a quantity of dried *S.trilobata* leaves into a graduated measuring cylinder. The volume is obtained by continuous tapping done manually, until constant value is noted and the bulk density is calculated.

**8. Phytochemical components in Daisy Tea:**

Total **flavonoid** content was measured by the aluminium chloride colorimetric assay. **Alkaloid** content was determined using U/V Visible spectrophotometer. The concentration of phenolics in the sample was determined using spectrophotometric method and Folin-Ciocalteu assay method was used for the determination of the total **phenol compounds**. **Carbohydrate** content is found using the colour change from green to dark green at 630 nm using Anthrone reagent.

**9. Diffusion rate:**

The time required for the particles to diffuse per unit area is considered as the diffusion rate of dip tea bags.

**10. Anti-scavenging activity:**

DPPH Assay was performed on the ethanolic extract of *S.trilobata* and the scavenging activity was determined using UV-V spectrophotometer.

**11. Detection of Microbes:**

Horizontal Method for Enumeration of Coagulase-Positive *Staphylococci aureus* was applied for their detection in the 15g of dried *S.trilobata* powder using Baird-Parker Agar Medium. The number of colony-forming units (CFU) of -glucuronidase-positive *Escherichia coli* per gram or per millilitre of sample is calculated. Detection of **yeast and mould** using Yeast Extract-Destmse-Chloramphenicol-Agar medium and the count was then calculated by the interpretation of formula. Thereafter, *Salmonella* detection was carried out by four successive stages: pre-enrichment in non-selective liquid medium, enrichment in selective liquid media, plating out and recognition, confirmation.

## 12. Sensory analysis:

A Sensory analysis based on consumer acceptance of Daisy Tea is conducted using the 9-point Hedonic scale in the Food analysis Laboratory of Avinashilingam institute. A five member panel including students was set up and the survey was conducted.

## III. RESULTS AND DISCUSSION

### 1. Biochemical analysis:

The analysis of the product was conducted based on the parameters such as Ash content, pH and acidity, Bulk density. The results are given as follows:

S NO	PARAMETERS	RESULTS
1	Ash content	6.8%
2	pH @ 10% solution	5.9
3	Acidity	3.24%
4	Bulk density	0.24 g/ml

**Table 3.1:** Biochemical Analysis

The results of ash content, pH, acidity and bulk density satisfies the customary values of food standard, thus ensuring the quality of the product.

### 2. Moisture content:

The moisture content of dried *S. trilobata* leaves is observed for a period of 10 days accurately on 0<sup>th</sup> day, 5<sup>th</sup> day and 10<sup>th</sup> day. The results are tabulated below:

S NO	PARAMETERS	RESULT
1	Moisture – 0 <sup>th</sup> day	4.39%
2	Moisture – 5 <sup>th</sup> day	6.39%
3	Moisture – 10 <sup>th</sup> day	8.24%

**Table 3.2:** Determination of Moisture Content

The observed moisture content on 10<sup>th</sup> day is found to be 8.24% which is less than the standard moisture content (13 -16%) . Therefore the shelf life of Daisy tea is achieved for a longer period.

### 3. Phytochemical analysis:

Phytochemical screening is conducted on dried *S.trilobata* leaves through which was found that the dried form showed a higher flavonoid content when compared to the natural form.

Therefore, drying results in the enhancement of phytochemical properties of Daisy tea. The results are tabulated as follows:

Phytochemical compounds	Dried <i>S.trilobata</i>
Flavonoids	10.6 mg Catechin/g
Alkaloids	6.3 mg Atropine/g
Phenolic Compounds	7.8 mg Gallic acid/g
Carbohydrates	685 mg Glucose/g

**Table 3.3:** Phytochemical screening

#### 4. Diffusion rate:

The Daisy tea bag with net weight 1 gram is dipped in 85-100°C water with varying volumes of 100 ml, 150 ml, 250 ml, naming each sample as A, B and C respectively. The Diffusion rate of the dip bag is 3-4 minutes, 5-6 minutes, and 7-8 minutes for A, B, and C respectively.



**A-100 ml      B-150 ml      C-250 ml**

**Fig 3.4:** Daisy tea in different volumes of water

#### 5. Phytochemical Screening:

Phytochemical compounds in the aqueous extract of *S.trilobata* leaves is tabulated as follows:

Phytochemical Compound	Aqueous extract of <i>S.trilobata</i> leaves
Flavonoid	+
Alkaloid	+
Phenolic compounds	+
Carbohydrates	+
Cholesterol	-
Tannins	+
Steroids	-
Saponins	-
Anthocyanins	+

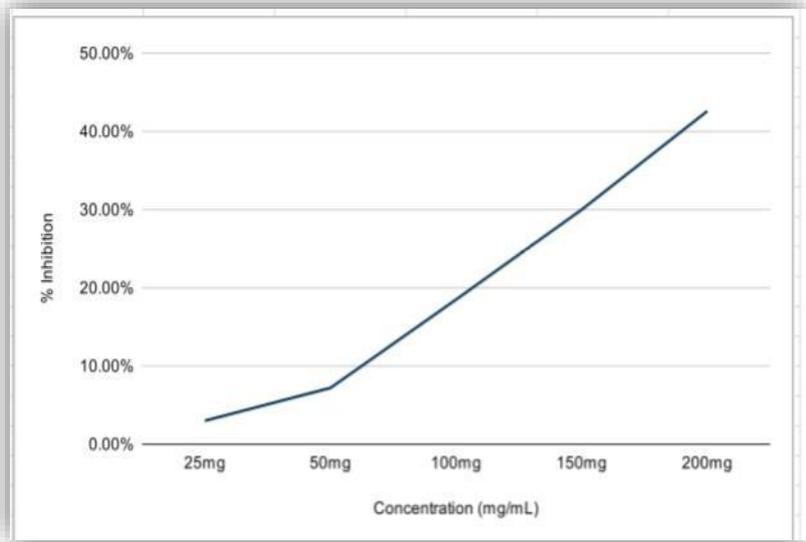
**Table 3.5:** Phytochemical screening of *S.trilobata***6. Packaging:**

Corn and wheat starch fermented into lactic acid, polymerised and spun into biodegradable teag bags is chosen as the packaging material. 1 gram of *S.trilobata* powder is packed in the tea bags. A cotton thread attached to the bag, bears the product tag.

**Fig 3.6:** Daisy Tea Dip Drink**7. Anti-scavenging activity:**

The free radical scavenging activity of the ethanolic extract of *S.trilobata* leaves is presented in the graph. The  $IC_{50}$  value is obtained from the linear regression equation that is constructed by the plotting a curve between inhibition and concentration of the sample. Thus the  $IC_{50}$  value of the leaves of *S.trilobata* is 236  $\mu\text{g/ml}$ , indicating the concentration of extract required scavenging 50% of the DPPH free radicals.

According to Mustarichie et al., antioxidant activity is categorized as a very powerful with  $IC_{50} < 50$  ppm, strong in the range of 50-100 ppm, moderate at 101-250 ppm, weak at 250-500 ppm, and classified as inactive at the  $IC_{50} > 500$  ppm<sup>[22]</sup>. Antioxidant activity of the *S. trilobata* leaves belongs to the moderate category.



**Fig 3.7:** % inhibition Vs concentration of *S.trilobata* extract

### 8. Detection of Microbes:

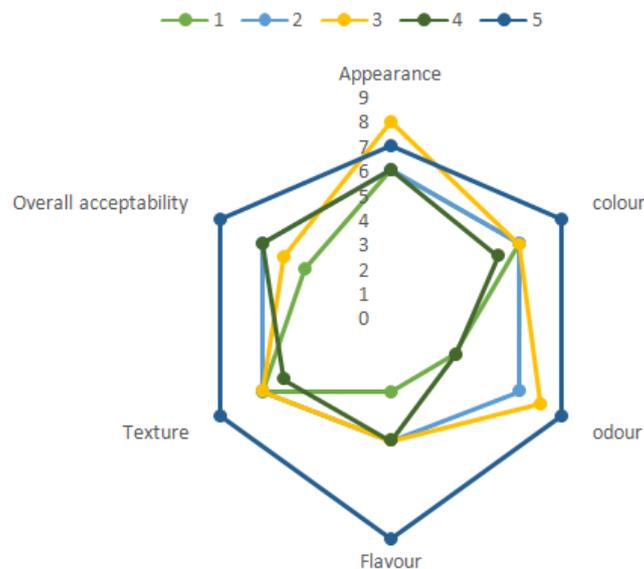
The following microorganisms are identified in the dried *S.trilobata* leaves.

Parameter	Result
<i>Staphylococcus aureus</i>	< 10cfu /g
<i>E. coli</i>	< 10cfu /g
Yeast and Mould	< 10cfu /g
<i>Salmonella spp</i>	Absent/g

**Table 3.8:** Microbial activity

### 9. Sensory Analysis:

The result of the sensory evaluation which was conducted on the formulated dip drink is consolidated and given as follows:



**Chart 3.9:** 9 Point hedonic radar chart

The flavour of Daisy tea from the sensory analysis resulted to be 5, that falls into the ‘neither like nor dislike’ category. Flavour addition and enhancement studies is further carried out according to the customer acceptable and global herbal drinks market.

#### IV. CONCLUSION

Singapore Daisy has exquisite medicinal properties that led to the preparation of Daisy Tea. The conceptualization of dip drink from *S.trilobata* leaves is due to its high anti-oxidant activity. The product evaluation for the presence of phytochemical compounds per gram showed acceptable values required for the dip drink. The microbial count was lesser with increased shelf life. From this study, it can be concluded that, the Daisy Tea can fulfil all the benefits which are proclaimed by providing the necessary medicinal properties like anti-oxidant and anti-inflammatory properties.

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