

## Phytochemical and cytotoxicity analysis of flowers of *Symphorema involucratum* (Lamiaceae)

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### Abstract

The present study investigates the secondary metabolites and toxicity of different extracts of *Symphorema involucratum* flowers. Qualitative phytochemical analysis revealed the presence of tannin, phenolic compounds, and reducing sugars in the aqueous, methanol, and ethanol extracts. Toxicity analysis using brine shrimp nauplii showed significant toxicity of the extracts, indicating their potential as therapeutic agents. The study suggests that *S. involucratum* flowers may be a valuable source of bioactive compounds with potential applications in formulation of drugs.

**Key words:** Bioactive compounds, medicinal values, plants

### Introduction

Medicinal plants have been an integral part of traditional medicine in India (Kumar and Jena, 2017; Kumar *et al.*, 2018; Kumar *et al.*, 2021; Mishra and Kumar, 2021; Das *et al.*, 2022; Kumar *et al.*, 2022; Kumar, 2022). The country is home to a rich diversity of medicinal plants (Kumar and Tripathy, 2017; Nayak and Kumar, 2023). These plants have been used to treat a wide

range of health problems (Das *et al.*, 2024; Sinha *et al.*, 2024; Laware *et al.*, 2024; Dimri *et al.*, 2024; Kumar, 2025). The importance of medicinal plants in India cannot be overstated, as they provide a vital source of healthcare for millions of people (Hebbbar *et al.*, 2024; Sethi *et al.*, 2024; Sharma *et al.*, 2024a; Mukherjee *et al.*, 2024; Bhat *et al.*, 2024; Palei *et al.*, 2024; Jena *et al.*, 2024; Sahu *et al.*, 2024; Kumar, 2024; Jena *et al.*, 2025).



Figure 1: Inflorescence of *S. involucreatum*

However, the increasing prevalence of antimicrobial resistance has become a major public health concern worldwide (Kumar *et al.*, 2013; Tripathy *et al.*, 2014; Kumar and Jena, 2014; Nayak *et al.*, 2015; Tripathy *et al.*, 2015; Murmu *et al.*, 2016; Kumar, 2017). The overuse and misuse of antibiotics have accelerated the emergence of resistant microorganisms, making it challenging to treat infections (Mancuso *et al.*, 2021). As a result, there is an urgent need to discover new antimicrobial agents that can effectively combat resistant microorganisms (Yao *et al.*, 2023). Medicinal plants offer a promising solution, as they have been shown to possess antimicrobial properties that can help combat infections (Abdallah *et al.*, 2023). Despite the rich diversity of medicinal plants in India, many species remain unexplored for their phytochemical and pharmacological properties. Phytochemical screening of unexplored plants is essential to identify new bioactive compounds that can be developed into novel medicines. This approach has led to the discovery of many life-saving medicines. In this context, the present study aims to investigate the availability of secondary metabolites of *Symphorema involucratum* flowers (Figure 1) and toxicity to *Artemia salina*.

## Materials and methods

The flowers of *S. involucratum* were collected from the different regions of Khordha district of Odisha, India (Figure 2). The plant species was identified by the authors. The Soxhlet extraction method was adopted using different solvents for phytochemical analysis (Figure 3-8; Devi *et al.*, 2024; Marndi *et al.*, 2024). An herbarium specimen is deposited to the Herbarium unit of Biodiversity and Conservation Lab of APRF, Cuttack, Odisha, India (Figure 9; Basole *et al.*, 2024; Jena *et al.*, 2025).

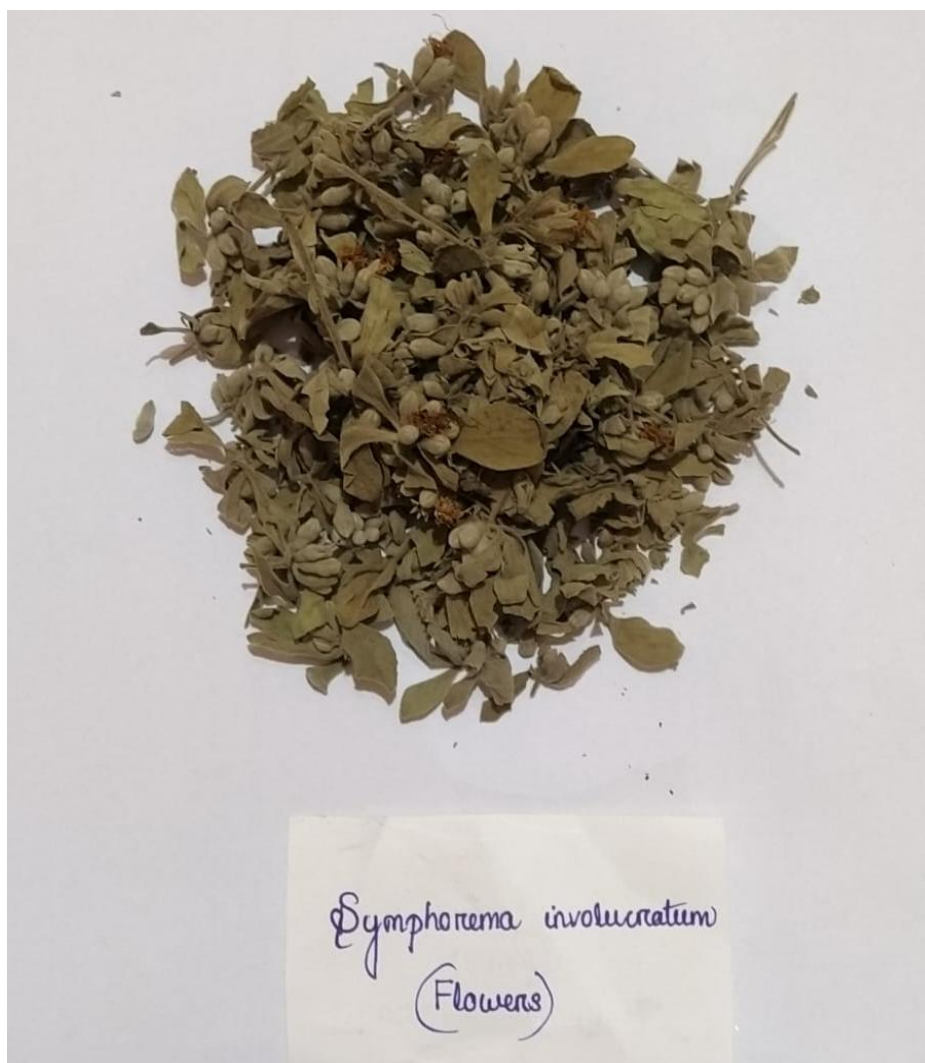


Figure 2: Collected flowers of *S. involucreatum* for experimental analysis

### **Qualitative phytochemical analysis**

Detection of nine secondary metabolites were conducted using standard methods (Sethi *et al.*, 2024; Sharma *et al.*, 2024b; Topno *et al.*, 2024).

**Test for tannin:** About 1 ml of the flowers extract was taken. 3-5 drops of 10% lead acetate solution were added to it. The formation of gelatinous precipitate confirmed the positive results for the presence of tannin.

**Test for saponin:** About 1 ml of the flowers extract was taken and 1 ml of distilled water was added and shaken well. The persistent froth formation confirmed the presence of saponin.

**Test for flavonoids:** About 1 ml of the flowers extract was taken. 2 ml of 2% NaOH solution and then dilute HCl were added to it. The colour initially turned to an intense yellow with NaOH solution and later became colourless. This colour-changing transformation confirmed the presence of flavonoids.

**Test for terpenoids:** About 1 ml of the filtrate was added with 6 drops of chloroform and placed in the water bath for a few minutes. Then 6 drops of concentrated sulphuric acid were added. The appearance of a reddish-brown interface confirmed the presence of terpenoids.

**Test for phenolic compounds:** About 1 ml of the flowers extract was taken and a few drops of 5% ferric chloride solution was added to it. The dark bluish-black appearance confirmed the presence of phenolic compounds.

**Test for reducing sugars:** About 1 ml of the flowers extract was taken and 2-3 drops of Fehling's solution A and B were added. Then kept in the water bath for some time. The presence of red-orange precipitate confirmed the presence of reducing sugar.

**Test for steroids:** About 1 ml of the flowers extract was taken. 1 ml of chloroform and 1 ml of concentrated sulphuric acid was added to it. The appearance of 2 phases with the upper red and lower yellow with green fluorescence confirmed the presence of steroids.

**Test for alkaloids:** About 1 ml of flowers extract was taken and 3 to 4 drops of Dragendroff's reagent was added. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

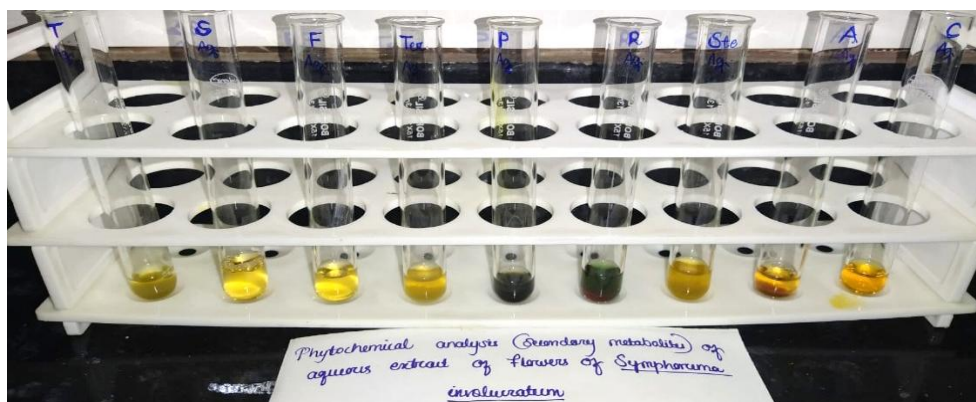


Figure 3: Detection of secondary metabolites of *S. involucratum* flowers using aqueous solvent

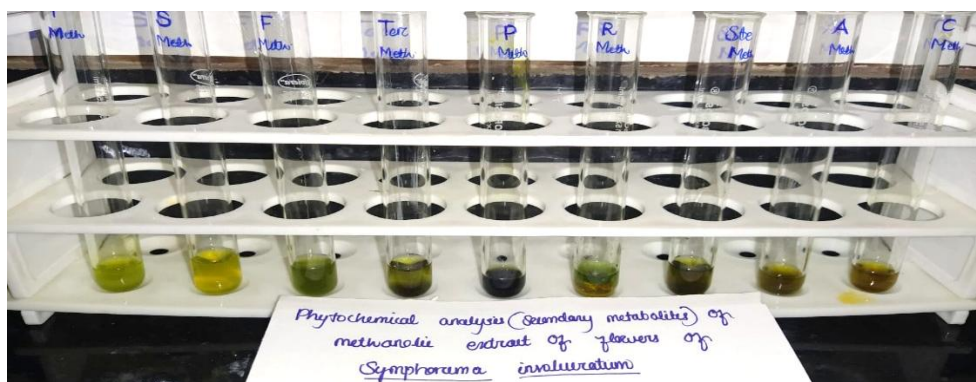


Figure 4: Detection of secondary metabolites of *S. involucratum* flowers using methanol solvent

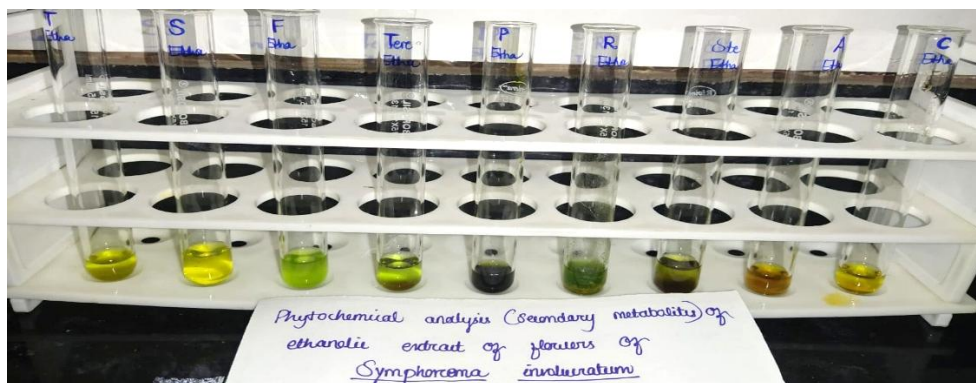


Figure 5: Detection of secondary metabolites of *S. involucratum* flowers using ethanol solvent

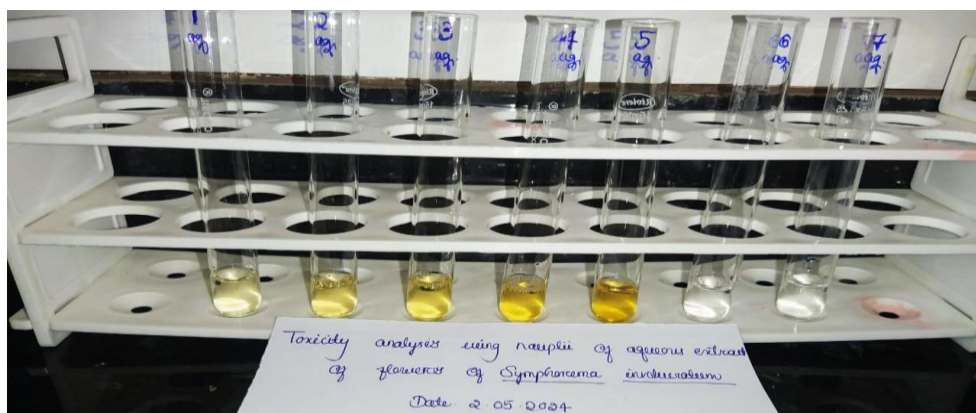


Figure 6: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *S. involucrellum* flowers

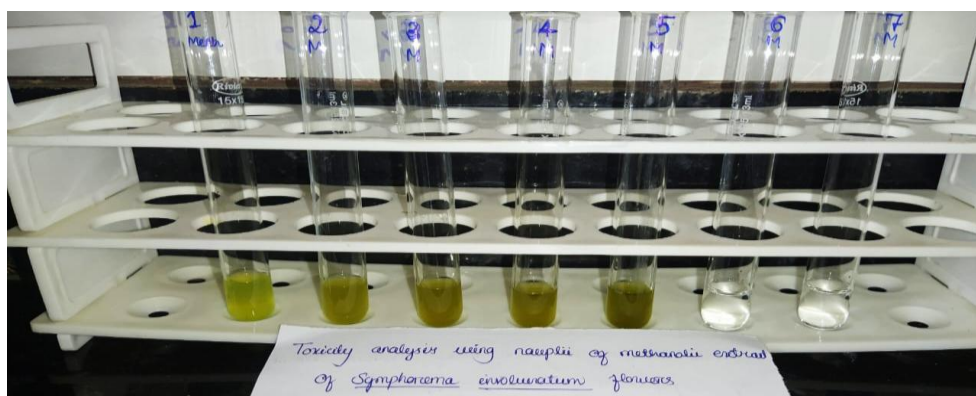


Figure 7: Toxicity analysis using Brine shrimp nauplii of methanolic extract of *S. involucrellum* flowers

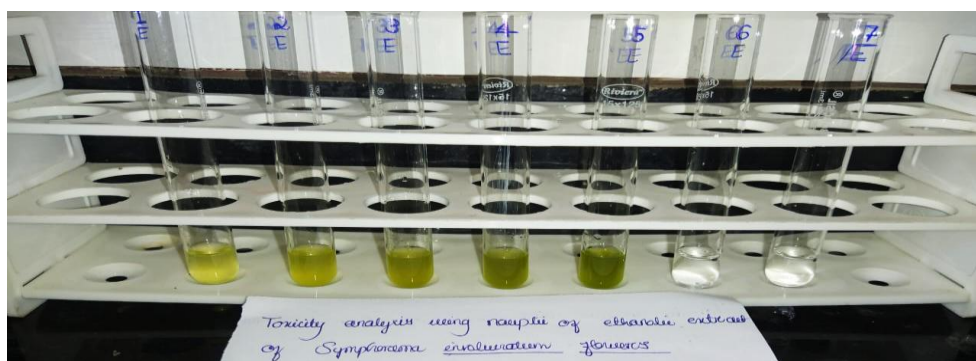


Figure 8: Toxicity analysis using Brine shrimp nauplii of ethanolic extract of *S. involucrellum* flowers

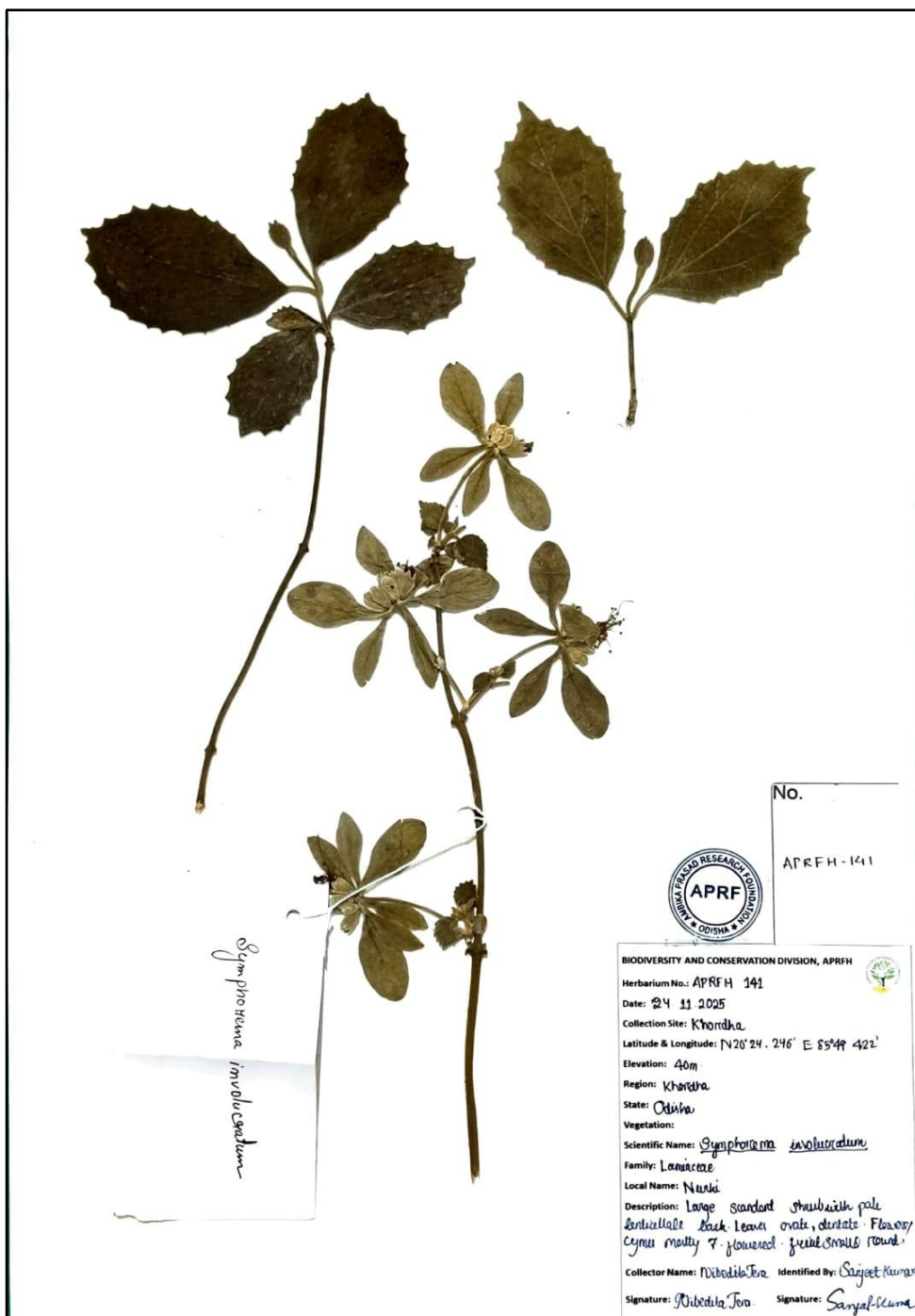


Figure 9: Herbarium specimen of *S. involucratum*

**Test for carbonyl compounds:** About 1 ml of the flowers extract was taken and 3 to 4 drops of 2,4-dinitrophenylhydrazine (DNPH) reagent was added to it. The yellow crystal formation confirmed the presence of carbonyl compounds.

### **Toxicity to *Artemia salina***

For toxicity analysis, hatching of Brine cysts was the initial process. Standardization of the optimum was done at 2.0, 3.5, and 5.0 % saline concentrations to check the optimum salinity for proper hatching of Brine shrimp (*Artemia salina*) cysts (Kumar *et al.*, 2012). The brine shrimp cysts are then incubated in 3.5% saline water with proper aeration, room temperature or between (28-35) ° C and light for 48 hours. Proper hatching could be observed within 24-48 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (15 mg/ml, 31 mg/ml, 47 mg/ml, 63 mg/ml and 79 mg/ml) of the *S. involucreatum* flowers extracts were taken and 3.5% saline water was used to make the volume 2 ml in each test tube. 1% DMSO (Dimethyl sulfoxide) was used to dissolve the crude extracts. Ten nauplii were selected and introduced to five test tubes of the extracts. Positive and negative controls were prepared using 3.5% saline water and vincristine sulphate (5 mg/ml) respectively with a volume of 2 ml. The live larvae are highly motile and thus differentiate from the unhatched cysts. The survivor nauplii were counted and analysed the death% (Kumar *et al.*, 2012).

### **Results and discussion**

The qualitative phytochemical analysis of the different extracts of *S. involucreatum* flowers revealed the presence of several bioactive compounds. Tannin, phenolic compounds, and reducing sugars were detected in all three

extracts (aqueous, methanol, and ethanol), indicating that these compounds are widely distributed in the plant (Table 1). The presence of these compounds suggests that *S. involucratum* flowers may possess antimicrobial, antioxidant, and anti-inflammatory properties, which could be useful in the development of new medicinal products (Table 3). The toxicity analysis using brine shrimp nauplii showed that all three extracts (aqueous, methanol, and ethanol) exhibited significant toxicity against the nauplii. The results indicate that the extracts are toxic at concentrations of 15-79 mg/ml, with the methanol extract showing the highest toxicity (Table 2). These results suggest that the extracts of *S. involucratum* flowers may have potential as therapeutic agents. The detection of these compounds suggests that *S. involucratum* flowers may possess antimicrobial, antioxidant, and anti-inflammatory properties, which could be useful in the development of new medicinal products (Figure 10).

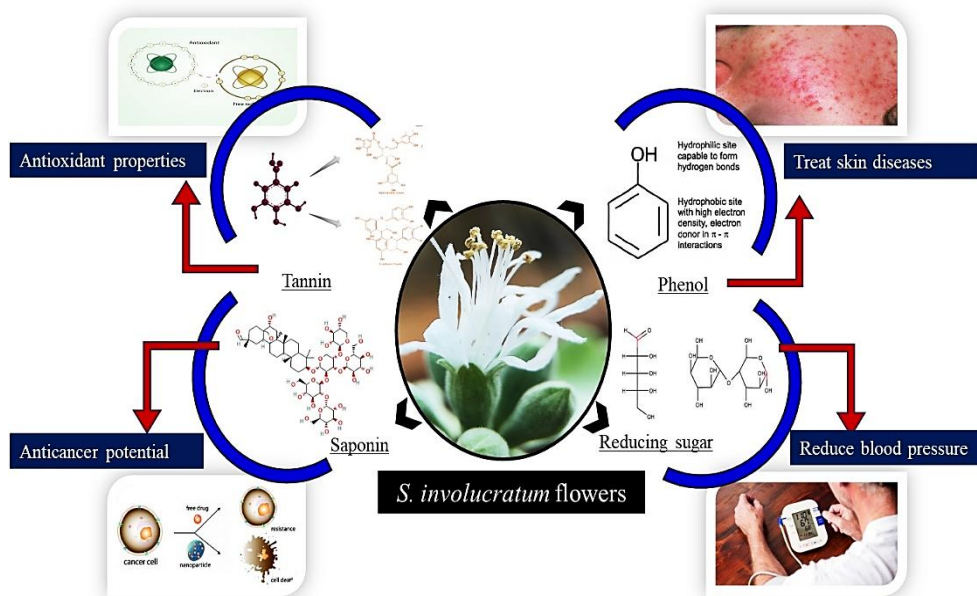


Figure 10: Pharmacological potentials of *S. involucratum* flowers

Table 1: Qualitative phytochemical analysis of different extracts of flowers of *S. involucratum*

| Bioactive compounds | Extracts |          |         |
|---------------------|----------|----------|---------|
|                     | Aqueous  | Methanol | Ethanol |
| Tannin              | +        | +        | +       |
| Saponin             | +        | -        | -       |
| Flavonoid           | -        | -        | -       |
| Terpenoid           | -        | -        | -       |
| Phenolic compounds  | +        | +        | +       |
| Reducing sugars     | +        | +        | +       |
| Steroids            | -        | -        | -       |
| Alkaloids           | -        | -        | -       |
| Carbonyl compounds  | -        | -        | -       |

(+: Positive, -: Negative)

Table 2: Toxicity analysis using Brine shrimp nauplii of different extracts of *S. involucratum* flowers

| Extracts | Concentration (mg/ml) | Initial number of Nauplii | Number of deaths of nauplii (after hours) |    |    |    |
|----------|-----------------------|---------------------------|-------------------------------------------|----|----|----|
|          |                       |                           | 1                                         | 2  | 3  | 4  |
| Aqueous  | 15                    | 10                        | 5                                         | 6  | 7  | 9  |
|          | 31                    | 10                        | 5                                         | 7  | 8  | 10 |
|          | 47                    | 10                        | 6                                         | 7  | 8  | 10 |
|          | 63                    | 10                        | 8                                         | 8  | 9  | 10 |
|          | 79                    | 10                        | 9                                         | 10 | 10 | 10 |
|          | 3.5% Saline           | 10                        | 0                                         | 0  | 0  | 0  |
|          | Vincristine sulphate  | 10                        | 9                                         | 10 | 10 | 10 |
| Methanol | 15                    | 10                        | 6                                         | 7  | 8  | 10 |

|         |                      |    |    |    |    |    |
|---------|----------------------|----|----|----|----|----|
|         | 31                   | 10 | 8  | 8  | 9  | 10 |
|         | 47                   | 10 | 8  | 9  | 10 | 10 |
|         | 63                   | 10 | 9  | 10 | 10 | 10 |
|         | 79                   | 10 | 9  | 10 | 10 | 10 |
|         | 3.5% Saline          | 10 | 0  | 0  | 0  | 0  |
|         | Vincristine sulphate | 10 | 10 | 10 | 10 | 10 |
| Ethanol | 15                   | 10 | 7  | 7  | 9  | 10 |
|         | 31                   | 10 | 8  | 8  | 10 | 10 |
|         | 47                   | 10 | 8  | 8  | 10 | 10 |
|         | 63                   | 10 | 9  | 9  | 10 | 10 |
|         | 79                   | 10 | 9  | 10 | 10 | 10 |
|         | 3.5% Saline          | 10 | 0  | 0  | 0  | 0  |
|         | Vincristine sulphate | 10 | 10 | 10 | 10 | 10 |

Table 3: Probable medicinal uses as per bioactive compounds

| Bioactive compounds | Medicinal uses                                                                                                                                 | Source                              |
|---------------------|------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| Tannin              | The presence of tannin in aqueous, methanol, and ethanol extracts indicates of having antioxidant properties.                                  | Fraga-Corral <i>et al.</i> , (2021) |
| Saponin             | The presence of saponin in aqueous extract tends to anticancer activities.                                                                     | Timilsena <i>et al.</i> , (2023)    |
| Phenolic compounds  | Phenolic compounds present in the aqueous, methanol and ethanol extracts have significant anti-inflammatory properties to treat skin diseases. | Rahman <i>et al.</i> , (2021)       |

|                 |                                                                                                      |                             |
|-----------------|------------------------------------------------------------------------------------------------------|-----------------------------|
| Reducing sugars | Reducing sugars present in aqueous, methanol and ethanol extracts indicate to reduce blood pressure. | Chen <i>et al.</i> , (2010) |
|-----------------|------------------------------------------------------------------------------------------------------|-----------------------------|

## Conclusion

In conclusion, the present study demonstrates the presence of bioactive compounds including tannin, phenolic compounds, and reducing sugars, in the extracts of *S. involucreatum* flowers. Furthermore, the toxicity analysis against brine shrimp nauplii suggests that these extracts possess potential therapeutic applications, making them promising sources for the development of novel medicinal agents.

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