

CHAPTER 2

Primary and secondary metabolites of Kusum seeds

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Abstract: *Schleichera oleosa*, commonly known as Kusum, belonging to the family Sapindaceae which has been used by the tribal communities for its medicinal properties. Kusum seed oils are used to treat wounds and different infectious diseases. The pharmacological activity of Kusum seeds is mainly due to the presence of various phytochemicals in them. The present study carried out on the seeds of *S. oleosa* using different extracts (aqueous, ethanol, acetone, and n-hexane) and reported the presence of phenols, tannins, terpenoids, and flavonoids. The findings suggest that Kusum seeds have a lot of therapeutic potential and could be used in the future for drug formulations.

Keywords: Health problems, urbanization, climate change, medicinal, phytochemicals

Introduction

Indigenous people use different types of seeds for various purposes. They have played an important role in human nutrition and health since long. They are the source of different types of secondary metabolites which are useful for sound health (Devi *et al.*, 2024). They have potential applications in the culinary and pharmaceutical aspects (Karthikeyan *et al.*, 2023). Metallic lifestyle, urbanization, and climatic changes are making a gap in the utilization of wild fruits and their seeds. They are also a source of livelihood in many forest areas and among the local communities of India (Bhatia *et al.*, 2013). They collect the seeds and consume them as food, extract oil, and sometimes store them for future food and medicinal agents (Mondal *et al.*, 2023). Documentation of such seeds and their medicinal uses is an important work for researchers in contemporary situations where the world is facing health and food problems. Keeping this in view, an attempt to document the primary and secondary metabolites of Kusum seeds. It is tree with dense foliage with thin

bark. Leaves paripinnate; leaflets opposite, sessile or very shortly petiolate, elliptic or oblong, entire, rarely repand, obtuse, acuminate or acute, glabrous. The flowers are yellowish and small. Stamens 4-8; filaments glabrous or slightly hairy. Drupe is ovoid and sharply pointed. Seeds are compressed brown (Saxena and Brahmam, 1994).

Methodology

Collection of the plant sample and preparation of extracts

The seeds of the Kusum plant were collected from the Badampahar Range of Rairangpur Forest Division (RFD), Mayurbhanj, Odisha, India in June-July 2024 (Figure 1). The plant species was identified by the senior author. The Soxhlet extraction method was adopted using different solvents for phytochemical analysis (Soni *et al.*, 2024; Marndi *et al.*, 2024; Figure 2). Detection of nine secondary metabolites was carried out using standard methods (Sharma *et al.*, 2024). An herbarium specimen (APRFH 106) was deposited in the Herbarium Unit of Biodiversity and Conservation Lab, Ambika Prasad Research Foundation, Odisha, India (Figure 3).

Qualitative phytochemical analysis

Detection of nine secondary metabolites were conducted using standard methods (Behera *et al.*, 2024).

Test for tannin

About 1 ml of the extract was taken. 3-5 drops of 10% lead acetate solution were added to it. The gelatinous precipitate formation confirmed the presence of tannin (Jena *et al.*, 2024).

Test for saponin

About 1 ml of the extract was taken and 1 ml of distilled water was added and shaken well. The formation of persistent froth was observed confirming the presence of saponin (Landge *et al.*, 2024).

Test for flavonoids

About 1 ml of the extract was taken. 2 ml of 2% NaOH solution and 3 to 4 drops of dilute HCL were added to it. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless. This change in colour confirmed the presence of flavonoids (Mety *et al.*, 2024).

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 H_2SO_4 were added. The reddish-brown interface confirmed the positive result for the presence of terpenoids (Dimri *et al.*, 2024).



Figure 1: Collection of seeds of *S. oleosa* in the field



Figure 2: Dried seed powder of *S. oleosa* for experimental work

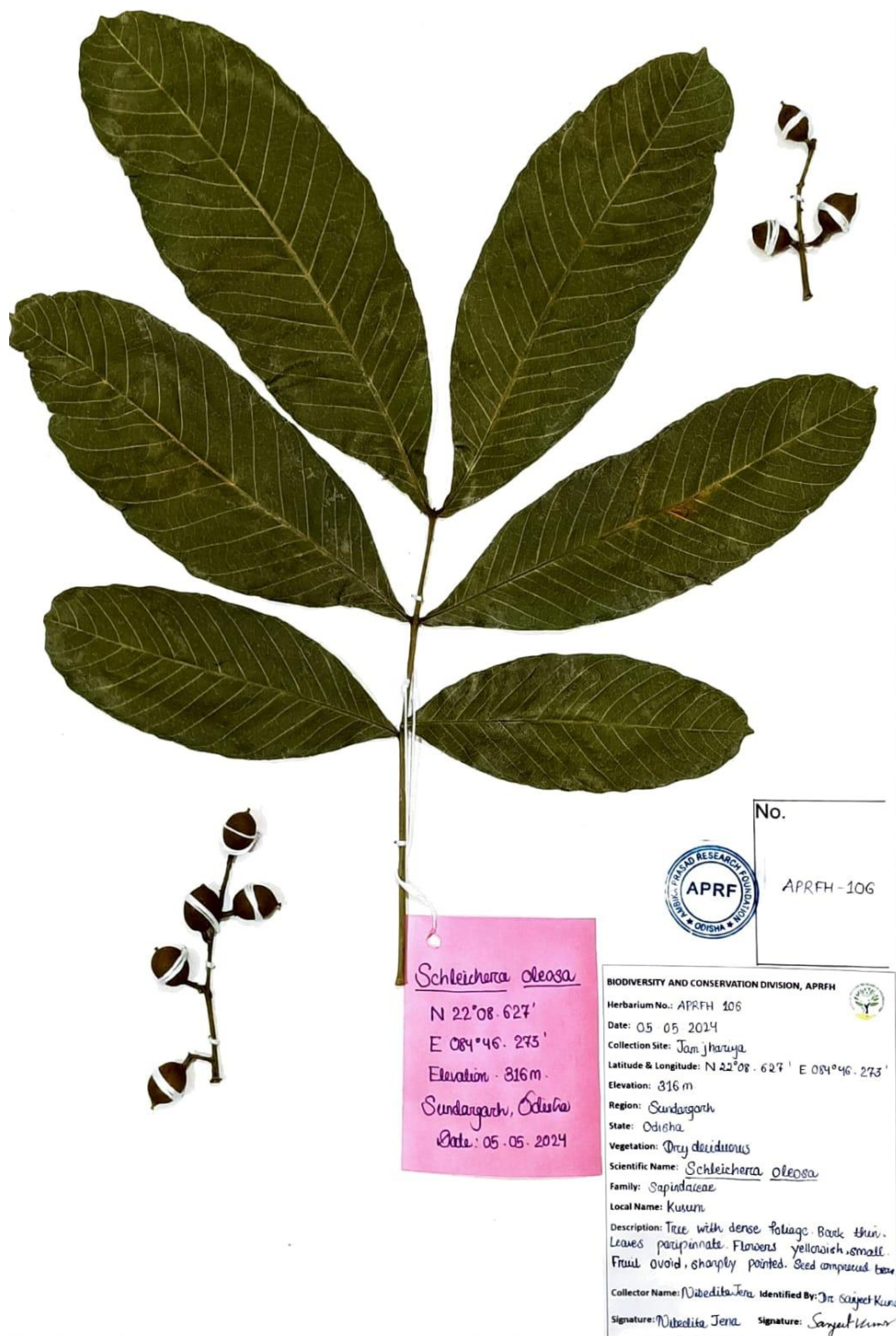


Figure 3: Herbarium specimen of *S. oleosa*

Test for phenolic groups

About 1 ml of the filtrate was taken. A few drops of 5% ferric chloride solution were added. The dark bluish-black provides the positive result of the test for phenolic groups (Topno *et al.*, 2024).

Test for reducing sugars

About 1 ml of the extract was taken and 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for some time. The presence of red-orange precipitate confirmed the presence of reducing sugar (Tripathi *et al.*, 2024).

Test for steroids

About 1 ml of the extract was taken. 1 ml of chloroform and 1 ml of concentrated sulphuric acid was added into it. The appearance of upper red and lower yellow with green fluorescence provides the presence of steroids (Vajha *et al.*, 2024).

Test for alkaloids

About 1 ml of the extract was taken and added 3 to 4 drops of Dragendorff's reagent. The formation of a reddish-brown precipitate confirmed the presence of alkaloids (Tallur *et al.*, 2024; Devi *et al.*, 2023).

Test for carbonyl compounds

About 1 ml of the extract was taken and added 3 to 4 drops of 2,4-Dinitrophenylhydrazine (DNPH) reagent. The formation of yellow crystal confirmed the presence of carbonyl compounds (Das *et al.*, 2024).

Detection of the primary metabolites were conducted using standard methods (Mohanty *et al.*, 2021)

Test for Protein

1 ml of the filtrate of extract was mixed with 2-5 drops of Millon's reagent and kept in the water bath for 30 minutes. The pink coloration indicates the presence of protein.

Test for carbohydrates

1 ml of the filtrate of extract was mixed with 2-5 drops of Fehling's solution A and B and kept in the water bath for 30 minutes. A reducing sugar reacts with Fehling's reagent in an alkaline medium to form orange-to-red precipitates. The appearance of this orange or red precipitate indicates the presence of carbohydrates.

Test for starch

To 1 ml of the filtrate of leaf extract, 2-3 drops of concentrated HCL were added and heated for 1-2 minutes. NaOH following Benedict's reagent was added to this solution and kept in the water bath. The change in coloration from bluish-green to red-orange indicates the presence of starch.

Test for sucrose

About 1 ml of the filtrate of leaf extract was mixed with 2-3 drops of iodine. The appearance of a blue-black colour indicates the presence of sucrose.

Test for lipid

About 1 ml of the filtrate of leaf extract was mixed with 2-5 drops of Sudan-III. The appearance of pink droplets indicates the presence of lipids.

Test for amino acid

1 ml of the filtrate of leaf extract was mixed with 5 drops of Ninhydrin reagent and kept in the water bath for 30 minutes. Ninhydrin is a powerful oxidizing agent that gives a purple-coloured product termed Rhuemann's purple.

Results and discussion

The qualitative phytochemical screening of secondary metabolites revealed that phenolic compounds were present in the aqueous, ethanolic, acetone, and n-hexane extracts of *S. oleosa* seeds. Tannin and reducing sugars were found in 3 extracts excluding the n-hexane extract while saponin was found in 3 extracts except acetone extract. Flavonoids and terpenoids were only found in the acetone extract of *S. oleosa* seeds (Table 1; Figures 4-7). The qualitative phytochemical screening of primary metabolites disclosed that carbohydrates and lipids were present in the aqueous, ethanolic, acetone, and n-hexane extracts of *S. oleosa* seeds. Starch was found in the acetone and n-hexane extracts; however, amino acids were only found in the ethanol extract of *S. oleosa* seeds (Table 2; Figures 8-11). Many researchers reported the pharmacological properties of the seeds of *S. oleosa*. Sahu *et al.*, (2022) worked on the seed extract of Kusum to reduce proliferation of breast cancer indicating that the Kusum seeds have anti-cancer and anti-metastatic activity.

Table 1: Phytochemical screening of secondary metabolites using different extracts of *S. oleosa* seeds

Bioactive compounds	Aqueous	Ethanol	Acetone	n-Hexane
Tannin	+ve	+ve	+ve	-ve
Saponin	+ve	+ve	-ve	+ve

Flavonoids	-ve	-ve	+ve	-ve
Terpenoids	-ve	-ve	+ve	-ve
Phenolic compounds	+ve	+ve	+ve	+ve
Reducing sugar	+ve	+ve	+ve	-ve
Steroids	-ve	-ve	-ve	-ve
Alkaloids	-ve	-ve	-ve	-ve
Carbonyl compounds	-ve	-ve	-ve	-ve

(+ve: positive; -ve: negative)

Table 2: Phytochemical screening of primary metabolites using different extracts of *S. oleosa* seeds

Primary metabolites	Aqueous	Ethanol	Acetone	n-Hexane
Protein	-ve	-ve	-ve	-ve
Carbohydrates	+ve	+ve	+ve	+ve
Starch	-ve	-ve	+ve	+ve
Sucrose	-ve	-ve	-ve	-ve
Lipid	+ve	+ve	+ve	+ve
Amino acid	-ve	+ve	-ve	-ve

(+ve: positive; -ve: negative)



Figure 4: Detection of secondary metabolites using aqueous extract of *S. oleosa* seeds

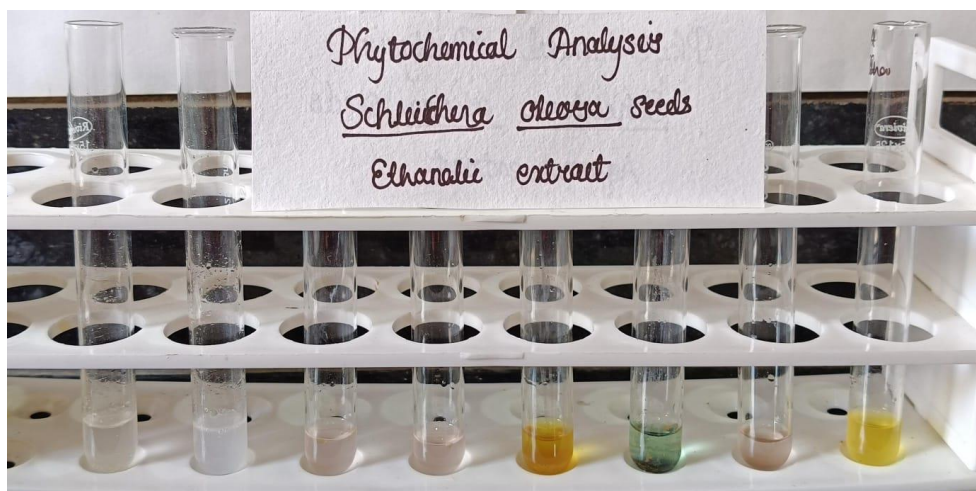


Figure 5: Detection of secondary metabolites using ethanolic extract of *S. oleosa* seeds



Figure 6: Detection of secondary metabolites using acetone extract of *S. oleosa* seeds



Figure 7: Detection of secondary metabolites using n-hexane extract of *S. oleosa* seeds

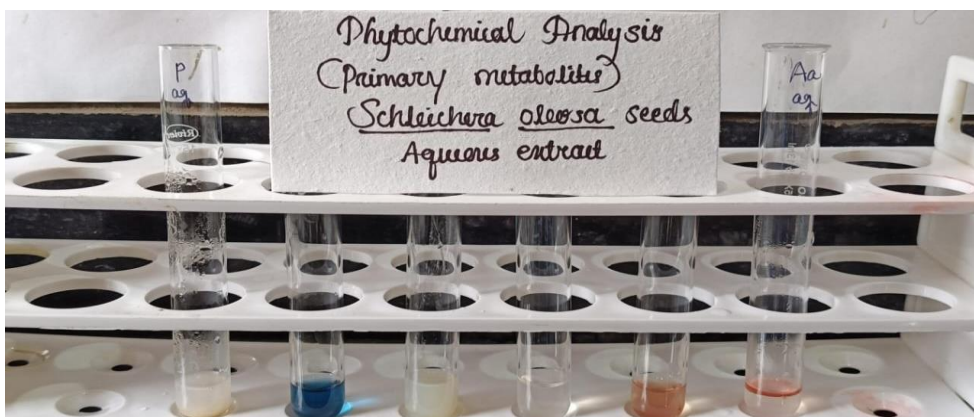


Figure 8: Detection of primary metabolites using aqueous extract of *S. oleosa* seeds



Figure 9: Detection of primary metabolites using ethanolic extract of *S. oleosa* seeds



Figure 10: Detection of primary metabolites using acetone extract of *S. oleosa* seeds

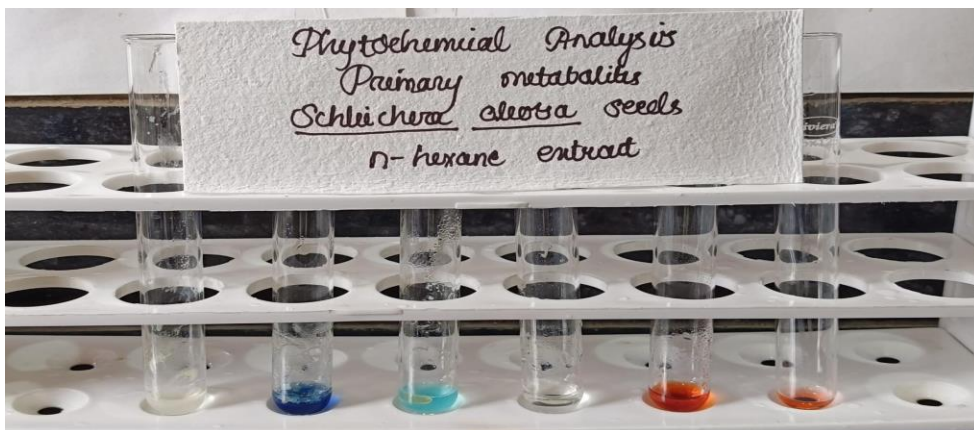


Figure 11: Detection of primary metabolites using n-hexane extract of *S. oleosa* seeds

Conclusion

The present study concludes that seeds of *S. oleosa* have a lot of pharmacological importance. The findings of phytochemical studies also support the uses of Kusum seeds in fighting different infectious diseases and reducing inflammation. The seeds have been used traditionally in herbal medications and this could be taken for further formulation of different drugs against present health problems using Kusum seeds.

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