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PHYTOCHEMICAL PROFILING AND EVALUATION ANTIBACTERIAL ACTIVITY OF *Osyris lanceolata*

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ABSTRACT

Osyris lanceolata is a medicinally important hemiparasitic large shrub or small tree with limited scientific investigation despite its ethnomedicinal significance. This study evaluated the phytochemical composition and antimicrobial activity of aqueous, methanol and ethanol leaf extracts of the study plant collected from Koraput, Odisha, India. Preliminary phytochemical screening revealed the presence of tannins, flavonoids, phenolics, steroids and terpenoids. Antimicrobial activity was assessed using the agar well diffusion method. Methanol and ethanol extracts exhibited the highest antibacterial activity, with maximum zones of inhibition of 15 mm at 100 mg ml^{-1}, while the aqueous extract showed comparatively lower activity (11 mm). The antimicrobial effect increased with extract concentration, indicating dose-dependent activity. The observed bioactivity is likely associated with the presence of phenolic and flavonoid compounds. These findings demonstrate that *O. lanceolata* is a promising source of bioactive phytochemicals with potential antimicrobial applications. Further studies on bioactive compound isolation and pharmacological validation are warranted to support its therapeutic development.

Keywords **:** Agar well diffusion, hemiparasitic plant, phytochemical.

Introduction

Osyris lanceolata Hochst. & Steud. commonly known as African sandalwood or East African sandalwood belonging to the family Santalaceae (Fig. 1), is a perennial evergreen hemiparasitic shrub or small tree widely distributed across Africa, the Mediterranean region, and parts of Asia, including India (Mishra and Kumar, 2026). The species has attracted considerable attention due to its ecological significance, aromatic wood and extensive ethnomedicinal applications. In India, particularly in Odisha, *O. lanceolata* occurs in dry deciduous forests and hilly regions of Koraput, where it forms an important component of local biodiversity and traditional healthcare systems (Mishra and Kumar, 2026; Ken, 2022). As a root hemiparasite, the species derives water and nutrients from neighbouring host plants while maintaining its own photosynthetic activity, thereby exhibiting unique ecological adaptations (Barradas *et al*., 2023). The species is highly valued for its fragrant heartwood and essential oils, leading to extensive harvesting and commercial exploitation. In many African countries, *O. lanceolata* provides substantial socio-economic benefits through its use in perfumery, traditional medicine and cultural practices (Mutisya *et al*., 2019; Mwangi *et al*., 2021; Ken, 2022). However, overexploitation, habitat degradation and poor natural regeneration have contributed to the decline of natural populations, resulting in its classification as an endangered species in several regions (Ken, 2022). Despite its conservation concerns, local communities continue to rely on the plant for the treatment of various ailments. Traditional medicinal systems across Africa and Asia employ different parts of *O. lanceolata*, including roots, bark,

leaves and stems, for the treatment of diarrhea, malaria, sexually transmitted infections, rheumatism, chest complaints, skin diseases and microbial infections (Shyaula, 2012; Ndegwa and Mbaria, 2025).

Fig. 1: *Osyris lanceolata* with its flowers and leaves

Ethnobotanical surveys have documented its widespread use in both human and livestock healthcare, reflecting the plant's broad therapeutic relevance and cultural significance (Mwangi *et al*., 2021; Mpolokeng *et al*., 2026). Such extensive traditional utilization suggests the presence of biologically active secondary metabolites that may contribute to its medicinal properties. Previous phytochemical investigations on the genus *Osyris* have revealed the presence of diverse bioactive compounds, including flavonoids, phenolics, tannins, terpenoids, saponins, alkaloids and glycosides (Shyaula, 2012). These classes of phytochemicals are well known for their antioxidant, antimicrobial, anti-inflammatory and cytotoxic activities. Research activities on *O. lanceolata* root bark extracts demonstrated significant radical scavenging activity and high phenolic content, indicating its potential as a source of natural antioxidants and therapeutic agents (Yeboah and Majinda, 2009). Bioactivity-oriented surveys of African medicinal plants have also highlighted the pharmacological significance of alkaloids and terpenoids occurring in species traditionally used for disease management (Babiaka *et al*., 2014). The emergence of antimicrobial resistance and the increasing incidence of cancer have intensified the search for novel bioactive compounds from medicinal plants. Natural products continue to serve as valuable sources of therapeutic agents due to their structural diversity and biological efficacy. Although *O. lanceolata* has a long history of ethnomedicinal use, comprehensive studies integrating phytochemical profiling and antimicrobial evaluations remain limited. In this context, the present study was undertaken to collect *O. lanceolata* from its natural habitat, authenticate the species using standard taxonomic procedures, investigate its phytochemical composition, and evaluate its antimicrobial activity. The findings are expected to enhance the understanding of the plant's therapeutic potential, provide scientific validation for its traditional medicinal uses and support its sustainable utilization and conservation.

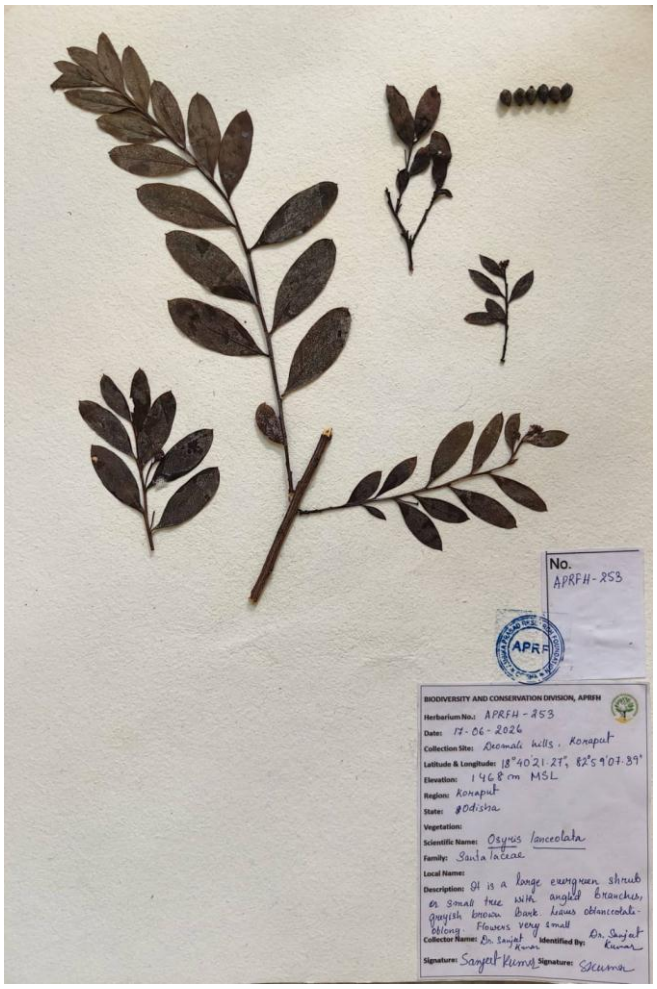

Fig. 2: Herbarium Specimen of *Osyris lanceolata*

Materials and Methods

The fresh plant parts of *O. lanceolata* were collected from Deomali hills of Koraput, Odisha and authenticated using standard taxonomic procedures. Representative herbarium specimen was prepared following standard herbarium techniques, which included pressing, drying, mounting and labelling (Fig. 2). The prepared herbarium specimens were preserved for future reference and taxonomic verification ((POWO 2026; Jain and Rao 1976; Saxena and Brahmam 1994). The collected plant parts were thoroughly washed, shade-dried and ground into a fine powder prior to extraction. Phytochemical extraction was carried out using the Soxhlet extraction method with solvents of varying polarity (Jena *et al*. 2024). The resulting extracts were subjected to qualitative phytochemical screening for selected secondary

metabolites using standard methods. (Kumar *et al*., 2013; Marndi *et al*., 2024).

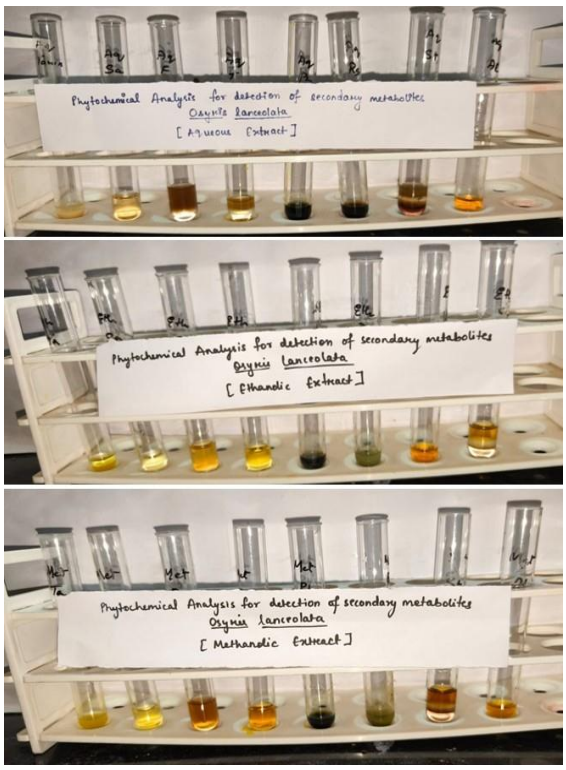

Fig. 3: Qualitative phytochemical analysis of *Osyris lanceolata*

Qualitative Phytochemical Analysis

The presence of major secondary metabolites, namely tannins, saponins, flavonoids, terpenoids, phenolic compounds, reducing sugars, steroids and alkaloids was determined using standard qualitative phytochemical tests (Fig. 3 & Table 1).

Lead Acetate Test for Tannins

1 ml of leaf extract was treated with 3-5 drops of 10% lead acetate solution. The formation of a gelatinous precipitate indicated the presence of tannins.

Froth Test for Saponins

1 ml of leaf extract was mixed with 1 ml of distilled water and shaken vigorously. The formation of persistent froth confirmed the presence of saponins.

Alkaline Reagent Test for Flavonoids

1 ml of leaf extract was mixed with 2 ml of 2% sodium hydroxide (NaOH) solution, followed by the addition of 3-4 drops of dilute hydrochloric acid (HCl). The appearance of an intense yellow colour that disappeared upon acidification indicated the presence of flavonoids.

Salkowski Test for Terpenoids

1 ml of leaf extract was mixed with 5-6 drops of chloroform and warmed in a water bath for a few minutes. Subsequently, 5-6 drops of concentrated sulphuric acid were added carefully. The formation of a reddish-brown interface confirmed the presence of terpenoids.

Ferric Chloride Test for Phenolic Compounds

1 ml of leaf extract was treated with a few drops of 5% ferric chloride solution. The appearance of a dark bluish-black colour indicated the presence of phenolic compounds.

Fehling's Test for Reducing Sugars

1 ml of leaf extract was mixed with two drops each of Fehling's solution A and Fehling's solution B and heated in a water bath. The formation of a brick-red to orange-red precipitate confirmed the presence of reducing sugars.

Salkowski Test for Steroids

1 ml of leaf extract was mixed with 1 ml of chloroform and 1 ml of concentrated sulphuric acid. The development of a red upper layer and a yellow lower layer with green fluorescence indicated the presence of steroids.

Dragendorff's Test for Alkaloids

1 ml of leaf extract was treated with 3-4 drops of Dragendorff's reagent. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Antibacterial activity

The antimicrobial activity of *O. lanceolata* extracts was evaluated using the Agar Well Diffusion method following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) with slight modifications. A fresh culture of *E. coli* was inoculated into sterile nutrient agar using the pour plate technique and allowed to solidify under aseptic conditions. Aqueous and ethanolic extracts of *O. lanceolata* were prepared at concentrations of 25, 50, and 100 mg/ml. Sterile cork borers were used to punch wells of 6 mm diameter into the agar medium. Subsequently, 100 µl of each extract concentration was carefully dispensed into the respective wells. A well containing 1 mg/ml kanamycin solution served as the positive control, while the corresponding extraction solvent was used as the negative control. The inoculated plates were incubated at 37°C for 24 h. Following incubation, the antimicrobial activity was assessed by measuring the diameter of the clear inhibition zones surrounding each well. All treatments were performed in triplicate, and the mean zone of

inhibition was recorded and expressed in (mm) (Owusu *et al*., 2021). The minimum inhibitory concentration (MIC) of the *O. lanceolata* extracts was determined using the broth dilution method. Serial dilutions of each extract were prepared to obtain concentrations ranging from 10 to 100 mg/ml. For each concentration, 100 µl of the plant extract and 50 µl of the bacterial inoculum were added to sterile nutrient broth tubes. Positive control tubes contained nutrient broth inoculated with the bacterial suspension, whereas negative control tubes contained only sterile nutrient broth. The tubes were incubated at 37°C for 24 h, after which they were examined for visible turbidity. The lowest concentration of the extract that showed no visible bacterial growth was recorded as the Minimum Inhibitory Concentration (MIC) (Owusu *et al*., 2021).

Results and Discussion

Field photographs of *Osyris lanceolata* were taken during the survey and herbarium specimens were prepared following standard botanical procedures for taxonomic authentication and permanent reference. The qualitative phytochemical screening of it revealed the presence of several bioactive secondary metabolites in all three solvent extracts. Tannins, flavonoids, terpenoids, phenols and reducing sugars were detected in the aqueous, methanolic and ethanolic extracts. Saponins were present only in the aqueous extract, while steroids were detected exclusively in the methanolic and ethanolic extracts, indicating that solvent polarity influences the extraction of specific phytochemicals (Table 1 & Fig. 4).

Table 1: Qualitative assessment of secondary metabolites in *Osyris lanceolata*

Solvent extracts	Phytochemicals present
Aqueous	Tannin, Saponin, Flavonoid, Terpenoid, Phenol, Reducing sugar
Methanol	Tannin, Flavonoid, Terpenoid, Phenol, Reducing sugar, Steroid
Ethanol	Tannin, Flavonoid, Terpenoid, Phenol, Reducing sugar, Steroid

The presence of tannins, flavonoids, phenols, and terpenoids suggests that *O. lanceolata* possesses significant antioxidant and antimicrobial potential. Tannins and phenolic compounds are known for their antioxidant and antibacterial activities, whereas flavonoids and terpenoids contribute to anti-inflammatory and antimicrobial properties. The detection of saponins in the aqueous extract may enhance antimicrobial activity through membrane disruption, while steroids in the methanolic and ethanolic extracts are associated with anti-inflammatory and antimicrobial effects. The antibacterial activity of *Osyris lanceolata* was assessed using the agar well diffusion assay for three different solvents i.e., aqueous, methanol and ethanol extracts. All extracts exhibited concentration-dependent antibacterial activity, with the zone of inhibition increasing as the extract concentration increased from 25 to 100 mg/ml.

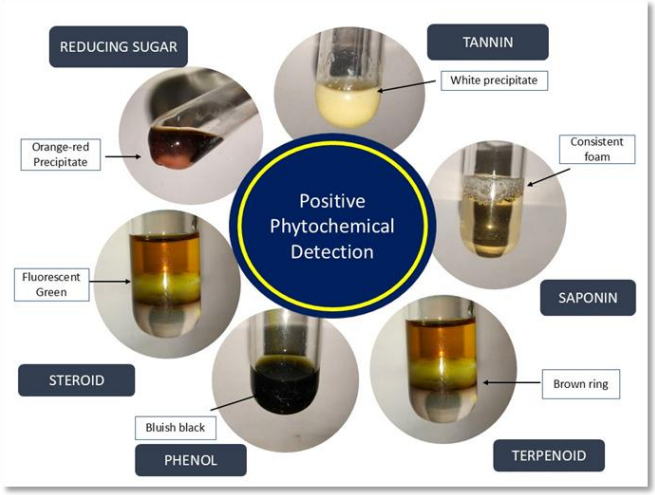

Fig. 4: Positive phytochemical detection in *O. lanceolata*

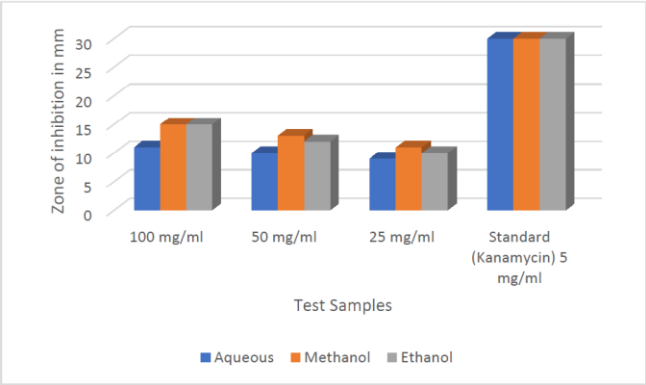

Fig. 5: Agar Well Diffusion Assay for three different solvent extracts of *O. lanceolata* in comparison with standard (Kanamycin)

Among the extracts, the methanol extract showed the highest antibacterial activity, producing an inhibition zone of 15 ± 0.0 mm at 100 mg/ml, followed by the ethanol extract (15 ± 0.2 mm), while the aqueous extract exhibited the lowest activity (11 ± 0.0 mm). A similar trend was observed at 50 mg/ml, where methanol (13 ± 0.2 mm) was more effective than ethanol (12 ± 0.0 mm) and aqueous (10 ± 0.1 mm), and at 25 mg/ml, with inhibition zones of 11 ± 0.1 mm, 10 ± 0.0 mm, and 9 ± 0.0 mm, respectively. The standard antibiotic, Kanamycin (5 mg/ml), produced a significantly larger inhibition zone (30 ± 0.0 mm) than all plant extracts. The superior antibacterial activity of the methanol and ethanol extracts may be attributed to

their higher extraction efficiency of bioactive phytochemicals such as phenolics, flavonoids, tannins and terpenoids, which possess well-documented antimicrobial properties (Fig. 5). The Minimum Inhibitory Concentration (MIC) of the methanol, ethanol and aqueous extracts against *Escherichia coli* was determined by the broth dilution method and was found to be 80 mg/ml, 90 mg/ml and 100mg/ ml respectively. Although the plant extracts were less effective than Kanamycin, their dose-dependent antibacterial activity demonstrates that *O. lanceolata* is a promising source of natural antimicrobial compounds and warrants further studies to isolate and characterize its active constituents.

Conclusion

O. lanceolata is a hemiparasitic plant found in specific regions of India, where it grows under distinct ecological and habitat conditions. The detection of bioactive phytochemicals, including tannins, flavonoids, phenolic compounds, steroids and terpenoids, indicates its considerable therapeutic potential, particularly for antioxidant and antimicrobial applications. Scientific investigation of the medicinal properties of this relatively underexplored parasitic plant could contribute to the discovery of novel bioactive compounds and support future drug development efforts, especially in addressing the growing global challenge of antimicrobial resistance.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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