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Research Article

Evaluation of phytochemical composition and biological activities of ethanol extract of bark of *Dialium ovoideum* Thwaites

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ABSTRACT

The present study aimed to investigate the biological activities of the ethanol extract of the bark of *Dialium ovoideum* Thwaites (DOEB), which is an endemic plant of Sri Lanka with various medicinal values. A powdered bark sample was subjected to soxhlet extraction using ethanol followed by solvent evaporation to obtain the bark extract. The phytochemical screening performed on DOEB revealed the presence of phenols, flavonoids, tannins, saponins, terpenoids and steroids. The total phenolic and flavonoid contents of the DOEB were measured as 233.4±22.7 mg GAE/g and 198.1±24.5 mg QE/g, respectively. The antioxidant activity of DOEB showed an EC_{50} of 68.5±6.3 µg/mL for the DPPH free radical scavenging assay and a significant ($p<0.05$) reductive potential compared to the ascorbic acid for the FRAP assay with a percentage reductive potential ranging from 5.7±0.8 to 79.2±2.2% across concentrations of 12.5 to 400 µg/mL. The modified L-DOPA method revealed the anti-tyrosinase activity of DOEB which is expressed as the IC_{50} of 1298.3±36.2 µg/mL. The human red blood cell stabilization assay by heat hemolysis resulted in an IC_{50} of 425.7±9.3 µg/mL. The agar-well diffusion assay was conducted to assess the anti-microbial activities against the *Staphylococus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* bacterial strains and the fungus, *Candida albicans*. The results exhibited that DOEB inhibited the growth of all three bacteria and fungi in different concentrations. Functional groups analysis of DOEB by FT-IR spectroscopy revealed the presence of alcohols, carboxylic acids, alkanes, alkenes, aliphatic esters, carbonyl and aromatic compounds. This study showed that DOEB has promising bioactivities based on the assays conducted which has a high potential to isolate bioactive compounds. Hence, DOEB may be employed as a prospective candidate in the food and pharmaceutical industries.

Keywords: *Dialium ovoideum*, ethanol extract of bark, phytochemical composition, biological activities, FT-IR spectroscopy

INTRODUCTION

Despite the advancements in modern medicine, medicinal plants continue to hold a significant role in the field of modern medicine (Weerasinghe and Deraniyagala, 2016). Traditional medicine involves the extraction of compounds from various parts of the plants, which offer a wide range of therapeutic properties (Sukmandari *et al.*, 2017). The use of plant-based products in developing novel and effective medications has become increasingly popular in the pharmaceutical industry, especially in treating non-communicable diseases such as cancer, diabetes, and inflammatory conditions (Yuan *et al.*, 2006). Therefore, combining conventional and contemporary approaches in

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the drug discovery process can yield better sources of active ingredients for disease treatments that have fewer adverse effects (Dhakar *et al.*, 2019). Many of the plants that are endemic to Sri Lanka are highly valuable for their medicinal properties and contain natural compounds that have bioactive properties.

Gal Siyambala botanically known as *Dialium ovoideum* Thwaites, is a species belonging to the *Dialium* genus in the Fabaceae family, found in the semi dry zone of Sri Lanka (Bulugahapitiya *et al.*, 2020b). Among the *Dialium* genus of 35 species, *D. ovoideum* Thwaites is the endemic species to Sri Lanka (Junior *et al.*, 2016). Trees have an average height of 20-30 m and bear whitish flowers and densely circular and flattened velvet fruits, which have a sweet acidic taste with edible pulp. The fruits of this plant are rich in vitamins and minerals.

According to traditional medicine, various parts of this plant have been used to treat a range of health issues. The leaves have been applied to shield wounds to protect them from germs and moisture, while the bark has been used to prepare antidote for snake bites. Furthermore, both the bark and leaves have been used to treat skin infections (Bulugahapitiya *et al.*, 2020a,b).

Previous studies are available on phytochemical composition, antioxidant properties, antimicrobial activity, and toxicity studies of the leaves of *D. ovoideum*. In the present study, an attempt has been made to investigate the phytochemical composition and biological activities of the ethanol extract from the bark of *D. ovoideum*.

MATERIALS AND METHODS

Preparation of the plant extract

Bark materials of *D. ovoideum* were collected from Gonagama area, Kurunegala district of Sri Lanka. The plant was identified and verified from Bandaranayake Memorial Ayurvedic Research Institute, Nawinna, Sri Lanka and the specimen was deposited under accession no. 4126 a. The bark materials were washed thoroughly with water and shade dried until they acquired a constant weight. Then materials were ground into a fine powder. A 65 g bark sample was subjected to Soxhlet extraction and extracted with 500 mL of ethanol for 6 h. The bark extract was obtained by filtering and concentrating the resulting extract using solvent evaporation. The powdered bark extract was stored at 4 ºC until further use and it was reassembled with organic solvents/buffer to obtain adequate concentrations of the extract for use in all studies.

Phytochemical screening

The ethanol extract obtained from the bark of *D. ovoideum* (DOEB) was subjected to phytochemical analysis using standard methods (Yadav *et al.*, 2014; Aththanayaka *et al.*, 2023). The experiments were conducted to determine the presence of phenols, flavonoids, tannins, saponins, terpenoids, and steroids.

Determination of the total phenolic content

The total phenolic content (TPC) of DOEB was determined by the Folin Ciocalteu (FC) method. Gallic acid was used as the reference standard. An aliquot of 0.5 mL of different concentrations of gallic acid (25-200 µg/mL) was mixed with 2.5 mL of 10% v/v FC reagent and allowed to stand in the dark for 10 min at room temperature. Na_2CO_3 (7.5% w/v, 2.0 mL) was added to the reaction mixture. This mixture was kept in the dark for 1 h at room temperature and the absorbance was measured at 765 nm. The extract was prepared in the same manner. The results were expressed as milligrams of gallic acid equivalents per dry weight of the plant extract (GAE/g) by using the standard curve (Thrikawala *et al.*, 2018).

Determination of the total flavonoid content

Total flavonoid content (TFC) was determined using $AlCl_3$ colourimetric method and quercetin was used as the standard. A volume of 0.5 mL of quercetin (12.5-800 µg/mL) was mixed with 2 mL of distilled water and 0.15 mL of 5% $NaNO_2$. The mixture was kept in the dark for 5 min. Then 0.15 mL of 10% $AlCl_3$ was added to the mixture and kept in the dark for 6 min. Thereafter, 1 mL of 1 M NaOH and 1 mL of distilled water were added to the mixture. The absorbance was measured at 510 nm. Using the standard curve, milligrams of quercetin equivalents per dry weight of the plant extract (QE/g) was obtained (Thrikawala *et al.*, 2018).

Determination of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The ability of DOEB to scavenge the DPPH radical was determined according to the method described by Ogu *et al*. (2013) with slight modifications. The various concentrations (12.5-400 µg/mL) of standard/plant extract (0.25 mL) were mixed with 0.05 mg/mL of DPPH solution (0.75 mL) and allowed to stand in the dark for 10 min at room temperature. The absorbance of the samples was measured at 517 nm. The blank was comprised of methanol instead of the sample and ascorbic acid was used as the reference standard. The percentage free radical scavenging activity (*I%)* was calculated as equation (1).

$$(I\%) = \left[\frac{Ac–As}{Ac}\right] \times 100 \quad \text{... (1)}$$

Where A_C is absorbance for the control and A_S is absorbance for the standard/plant extract. Using the plot of % free radical scavenging activity vs concentration, EC_{50} value was obtained.

Ferric-reducing power assay (FRAP)

The ferric reducing power assay was used for determining the capacity of antioxidants ability to reduce Fe^{3+} to Fe^{2+} (Ogu *et al.*, 2013). Each concentration of the standard/plant extract (1 mL) was mixed with 1 mL of phosphate buffer (pH=6.6) and 1% w/v potassium ferricyanide (1 mL). The reaction mixture was incubated at 50 ºC for 20 min. After incubation, the reaction mixture was allowed to cool and 10% w/v of trichloroacetic acid (1 mL) was added followed by centrifugation of samples at 3000 rpm for 10 min. The supernatant (1 mL) was mixed with 2 mL of distilled water and 1% w/v ferric chloride (0.2 mL). Reaction mixtures were stood for 10 min and the absorbance of the resultant solution was measured at 700 nm. Ascorbic acid was used as the reference standard for the assay. The percentage reducing potential (*I%)* was calculated as equation (2).

$$(I\%) = \left[\frac{As–Ac}{As}\right] \times 100 \quad \text{... (2)}$$

Where A_S is absorbance for the standard/plant extract and A_C is absorbance for the control. Using the plot of % reducing potential vs concentration, the reducing potential was compared with the standard at the tested concentration range (12.5-400 µg/mL).

Determination of anti-tyrosinase activity

Inhibition of tyrosinase activity of DOEB was carried out using the modified 3,4-dihydroxy-L-phenylalanine (L-DOPA) method (Song *et al.*, 2009; Baek *et al.*, 2021). Tyrosinase enzyme was extracted from *Agaricus bisporus*. Standard/plant extract (1.4 mL) was mixed with tyrosinase enzyme (0.3 mL) and incubated for 5 min at 25 ºC. Then L-DOPA (4 mM, 2.0 mL) was added and incubated again for 25 min at 25 ºC. The absorbance of the produced dopachrome was measured at 475 nm. Kojic acid (0.05-8 mg/mL) was used as the reference standard for this method. Control was prepared using phosphate buffer in place of standard/plant extract. The percentage inhibition (*I%*) of tyrosinase by kojic acid and plant extract was then calculated using equation (3).

$$(I\%) = \left[\frac{Ac–As}{Ac}\right] \times 100 \quad \text{... (3)}$$

Where A_C is absorbance for the control and A_S is absorbance for the standard/plant extract. Using the plot of % inhibition of tyrosinase enzyme vs concentration, IC_{50} value was obtained.

Determination of anti-inflammatory activity

Human red blood cell (HRBC) membrane stabilization assay was conducted to determine the anti-inflammatory activity of DOEB. A blood sample was collected from a healthy volunteer who had not taken any anti-inflammatory drug for 2 weeks prior to obtaining the sample. A volume of 5 mL of blood was collected and centrifuged at 3000 rpm for 20 min. The resulting supernatant was discarded and the pellet was washed using a volume of normal saline equal to the pellet volume and centrifuged at 3000 rpm for 10 min. This process was carried out until the yellowish colour of the supernatant went off and became clear (about three times). Then 1 mL of the pellets was mixed with 9 mL of normal saline and made the 10% v/v red blood cell suspension. After preparing the suspension, heat induced hemolysis was conducted for the samples. Standard/plant extract (1 mL) was mixed with 0.1 mL of 10% v/v red blood

suspension and incubated at 56 ºC for 30 min. Samples were cooled to room temperature and centrifuged at 3000 rpm for 10 min. The absorbance of the supernatants was measured at 560 nm (Kuganesan *et al.*, 2017; Sarveswaran *et al.*, 2017). Aspirin was used as the reference standard. Red blood cell suspension and distilled water were used as the control and the blank respectively. Percentage inhibition (*I%*) of hemolysis was calculated using equation (4).

$$(I\%) = \left[\frac{\text{Ac–As}}{\text{Ac}}\right] \times 100 \quad \text{... (4)}$$

Where A_C is absorbance for the control and A_S is absorbance for the standard/plant extract. Using the plot of % inhibition of hemolysis vs concentration, IC_{50} value was obtained.

Determination of antimicrobial activity

Streptococcus aureus (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebisella pneumoniae* (ATCC 13883) and *Candida albicans* (ATCC 10231) pure clinical isolates were obtained from Medical Research Institute, Colombo, Sri Lanka. The antimicrobial activity of the plant extract was determined using agar well diffusion method with slight modifications (Soumia *et al.*, 2014). The sensitivity of different microorganisms was determined by the measurement of the inhibition zone diameter by well diffusion method. The Mueller-Hinton agar and Potato Dextrose agar were used for the bacterial isolates and fungal isolates respectively. The agar medium was prepared and poured into sterile petri dishes. The microbial suspensions were prepared by inoculating microorganisms from subcultures of *Streptococcus aureus*, *Escherichia coli*, *Klebisella pneumoniae* and *Candida albicans* into saline using a wire loop and the microbial suspensions were compared with the 0.5 McFarland turbidity standard. Wells were bored in the microorganisms-seeded plates with a sterile cork borer (8 mm diameter). Each well was loaded with 50 µL of plant sample (100-0.05 mg/mL), gentamicin (2 mg/mL) and ciprofloxacin (2 mg/mL) as positive controls for bacteria and fungi, respectively, and dimethylformamide (DMF) as negative control. The inoculated plates were kept for 1 h to allow the sample to diffuse properly into the agar and they were incubated at 37 ºC for 24 h. The sensitivity of the organisms to the extracts was determined by measuring the diameter of the zone of inhibition around the wells.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectroscopic analysis of DOEB was performed at a 500 to 4000 cm^{-1} scan range and numerical data were plotted using the OriginPro. 9.0 software.

Statistical analysis

All tests were performed in triplicate and data were presented as mean ± standard deviation (SD). OriginPro. 9.0 software was used for plotting graphs and the statistical analysis was conducted using IBM SPSS statistics 21. Values were considered to be statistically significant when $p \leq 0.05$.

RESULTS AND DISCUSSIONS

The phytochemical screening of DOEB revealed the presence of phenols, flavonoids, tannins, saponins, terpenoids and steroids using standard methods. The quantitative determination of phenols and flavonoids of DOEB exhibited comparatively high quantities of phenols and flavonoids equivalent to gallic acid and quercetin standards respectively. DOEB exhibited values of 233.4±22.7 mg GAE/g and 198.1±24.5 mg QE/g for the total phenolic and total flavonoid content which were comparatively high quantities.

Figure 1 demonstrated the antioxidant activity of DOEB using the DPPH radical scavenging assay, resulted with an EC_{50} value of 68.5±6.3 µg/mL, compared to ascorbic acid (32.0±2.1 µg/mL). DOEB (25-400 µg/mL) exhibited significant free radical scavenging activity that increased with the rising concentration, resulting in a higher percentage scavenging activity (85.5±2.1%) at 400 µg/mL compared to ascorbic acid (90.8±0.4%).

The DPPH radical scavenging assay demonstrated the ability of the antioxidants present in DOEB to donate electrons, thereby neutralize the radical to its corresponding hydrazine (Amabye, 2015; Dissanayake *et al.*, 2018).

The antioxidant activity of DOEB was further evaluated by comparing its reducing power to that of the ascorbic acid standard, assessed using the FRAP assay (Figure 2). DOEB exhibited a reducing potential ranging from 5.7±0.8 to 79.2±2.2% across concentrations of 12.5 to 400 µg/mL,

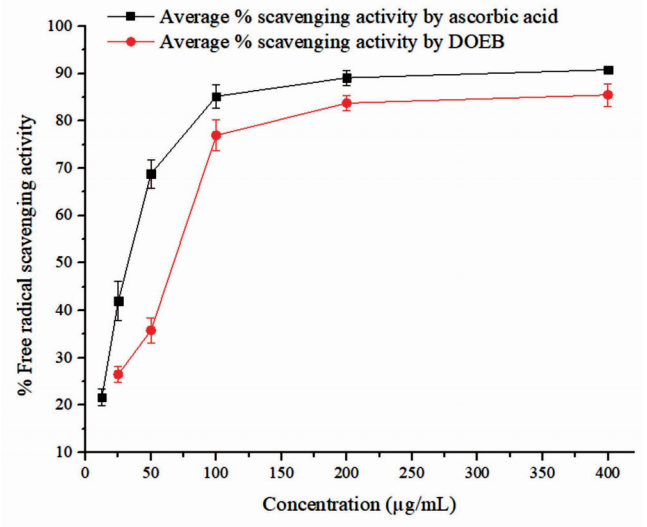

Figure 1: The percentage free radical scavenging activity of DOEB in comparison to the ascorbic acid standard at different concentrations

indicating a significant ($p<0.05$) reductive potential compared to ascorbic acid (17.5±1.6% to 90.9±1.1%).

The FRAP assay is based on the principle that higher absorbance in the reaction mixtures corresponds to increased antioxidant activity. The results indicated that DOEB contain antioxidants that effectively reduce Fe^{3+} to Fe^{2+}, demonstrating their reducing power (Saeed *et al.*, 2012; Uduwela *et al.*, 2019).

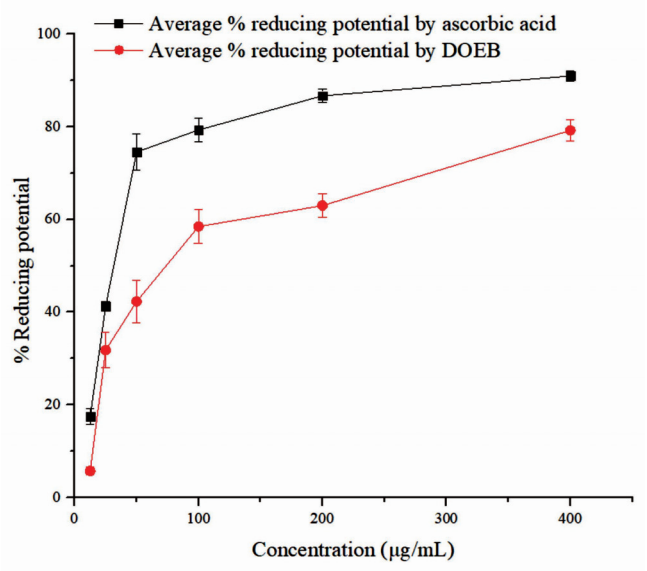

Figure 2: The percentage reducing potential of DOEB in comparison to the ascorbic acid standard at different concentrations

When comparing the antioxidant activity of DOEB with published findings of the same species, it was observed that DOEB exhibited notably high antioxidant activity compared to leaves of *D. ovoideum* (Bulugahapitiya *et al.*, 2020b). Since both extracts contained similar phytochemicals, this enhancement in DOEB can be attributed due to the presence of high contents of phenolic compounds, mainly flavonoids.

The anti-tyrosinase activity of DOEB is quantified by its IC_{50} value, which was determined to be 1298.3±36.2 µg/mL compared to kojic acid (IC_{50} value of 139.3±22.3 µg/mL) (Figure 3). Although the IC_{50} value was slightly higher than that of the kojic acid standard, it still indicated a significant anti-tyrosinase activity. DOEB exhibited notable percentage inhibition of the tyrosinase enzyme similar to the standard at the highest tested concentration, with an inhibition of 73.3±2.0% (kojic acid = 78.7±1.6%).

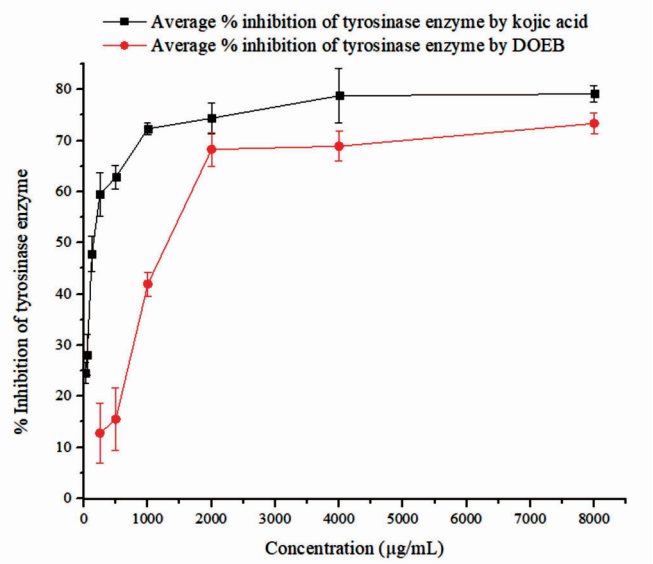

Figure 3: The percentage inhibition of tyrosinase enzyme by DOEB in comparison to the kojic acid standard at different concentrations

Tyrosinase inhibition can be achieved with bioactive compounds such as flavonoids, which directly block tyrosinase activity in melanogenesis, thereby exhibiting skin depigmentation properties. Moreover, the ability of flavonoids to bind with copper and act as antioxidants further contributes to their effectiveness in blocking the action of the tyrosinase enzyme (Dolorosa *et al.*, 2019). DOEB contained high content of flavonoids and many

phytoconstituents, further enhances these beneficial properties.

While scientific evidence regarding natural tyrosinase inhibitors within the genus *Dialium* remains limited, research has been conducted to identify tyrosinase inhibitors in species within the Fabaceae family, such as *Tamarindus indica*. The leaves of *T. indica* have been utilized in the development of topical applications, highlighting their anti-tyrosinase activity (Prabowo *et al.*, 2021).

This investigation aims to uncover new finding, stimulating interest in the development of novel, safe and effective natural tyrosinase inhibitors. The tyrosinase enzyme inhibition assay revealed the ability of DOEB to inhibit melanin synthesis, which is significant for addressing hyperpigmentation issues.

In the assessment of anti-inflammatory activity using the heat induced hemolysis assay, findings demonstrated a dose-dependent relationship. This implies that the percentage inhibitions increased proportionally with the concentration of the extract. Figure 4 depicts the anti-inflammatory activity of DOEB compared to aspirin standard. The obtained IC_{50} value for DOEB was 425.7±9.3 µg/mL, whereas the standard exhibited IC_{50} value of 162.0±7.9 µg/mL. While DOEB exhibited a higher IC_{50} value compared to standard, it demonstrated significantly increased percentage inhibitions of 72.4±2.4% and 77.1±2.6% at concentrations of 800 and 1600 µg/mL, respectively. This indicates enhanced anti-inflammatory activity at higher concentrations compared to aspirin standard, which showed inhibitions of 62.4±3.4% and 65.8±2.0% at the same concentrations.

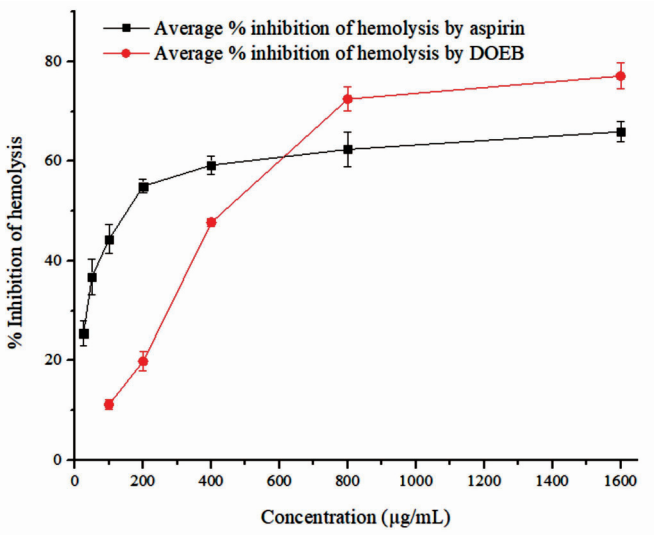

Figure 4: The percentage inhibition of hemolysis by DOEB in comparison to the aspirin standard at different concentrations

The HRBC membrane stabilization assay is an in vitro method utilized to assess the ability of compounds/extracts to prevent or reduce hemolysis, which is the rupture of red blood cells and release of hemoglobin into the surrounding medium (Gulnaz *et al.*, 2014). Phytochemicals such as flavonoids and steroids exhibit the capacity to interact with membrane components, thereby stabilizing the cell membrane. Steroids influence membrane fluidity and stability by directly interacting with membrane lipids (Rathee *et al.*, 2009; Patel *et al.*, 2015) . The presence of these phytochemicals in DOEB associated with significant anti-inflammatory properties.

A recent research has demonstrated the anti-inflammatory activities of different plant parts of various species in the genus *Dialium* by utilizing multiple anti-inflammatory assays. Among them, the stem bark of *D. guineense* exhibited potent anti-inflammatory activity with a low IC_{50} value of 0.15±0.01 µM compared to dexamethasone standard (4.31±1.45 µM) assessed through NO assay (Gnansounou *et al.*, 2019). The findings of the present study have unveiled the anti-inflammatory potential of DOEB, substantiating the evidence from existing literature with a significant ($p<0.05$) anti-inflammatory activity compared to the standard.

The mean diameter zone of inhibition was tested with different concentrations of DOEB extracts (100 mg/mL, 25 mg/mL, 5 mg/mL, 0.5 mg/mL and 0.05 mg/mL) against the diverse tested microorganisms. The results of this study revealed that DOEB exhibited inhibitory effects on all three bacteria and the fungal isolate compared to the positive controls, gentamicin and ciprofloxacin respectively (Table 1). The results of the inhibition zone diameters of DOEB revealed that *K. pneumoniae* was the most sensitive isolate, with an inhibition zone diameter of 15.0±0.8 mm at a concentration of 0.05 mg/mL while *E. coli* showed the least sensitivity with an inhibition zone diameter of 12.3±0.6 mm at a concentration of 25 mg/mL, which was comparable to the gentamicin standard. DOEB showed an inhibition zone diameter in a range of 12.3±1.0 to 22.8±1.0 mm against the fungus *C. albicans* in the concentration range of 5 to 100

Table 1: Antimicrobial activities of DOEB expressed as inhibition zone

Microorganism	Mean diameter zone of inhibition ± SD (mm)						
	Ethanol bark extract					Reference drug	
	100 mg/mL	25 mg/mL	5 mg/mL	0.5 mg/mL	0.05 mg/mL	Gentamicin (2 mg/mL)	Ciprofloxacin (2 mg/mL)
K. pneumoniae	NT	NT	NT	17.8±0.5	15.0±0.8	36.0±0.8	NT
S. aureus	14.5±0.7	13.3±0.6	NI	NI	NI	32.5±0.6	NT
E. coli	13.5±0.7	12.3±0.6	NI	NI	NI	30.8±1.0	NT
C. albicans	22.8±1.0	15.5±0.6	12.3±1.0	NI	NI	NT	43.0±2.2

NT: Not tested, NI: No inhibition

mg/mL compared to ciprofloxacin standard (inhibition zone diameter of 43.0±2.2 mm). According to the present study, all four isolates revealed a concentration dependent diameter zone of inhibition at different concentration ranges. The bacterial isolate *K. pneumoniae* demonstrated the highest sensitivity among the tested isolates. The least sensitivity was observed in *E. coli*.

Upon comparing the antimicrobial activity of DOEB with reported data of the same species, it was explored that different extracts of leaves of *D. ovoideum* exhibited strong anti-bacterial activity against *S. aureus* (Bulugahapitiya *et al.*, 2020 a). Moreover, a study conducted by Olajubu et al. (2012) showed antimicrobial effects of the stem bark of *D. guineense* against *S. typhi, S. aureus* and *C. albicans*.

The relationship between DPPH radical scavenging activity with anti-tyrosinase and anti-inflammatory activities of DOEB was examined. The strength of correlation between variables was assessed using Pearson's correlation coefficient (r), which ranges between -1.00 and +1.00. The results given in Table 2, displayed a very strong positive linear correlation (r=0.971, p=0.006, R^2=0.943) between the DPPH radical scavenging activity and anti-inflammatory activity of DOEB. Furthermore, the relationship between the DPPH radical scavenging activity and anti-tyrosinase activity showed a strong positive correlation (r=0.864, p=0.059, R^2=0.746). This relationship indicated that the anti-inflammatory and anti-tyrosinase activities of DOEB may be associated with its free radical scavengers.

Table 2: Correlation between DPPH radical scavenging activity with anti-tyrosinase and anti-inflammatory activities in DOEB

	Anti-inflammatory	Anti-tyrosinase activity
DPPH radical scavenging activity	0.971**	0.864

**Correlation is significant at the 0.01 level (2-tailed)

The FT-IR spectrum was utilized to determine the functional groups of the active compounds in DOEB, based on the peak values in the IR region (Figure 5). A board peak was observed at 3240 cm^{-1}, indicating a strong vibration of the free hydroxyl group of phenol (O-H stretch). Another intense peak, observed at 1604 cm^{-1}, which corresponds to a strong C=O stretch. Furthermore, the peak at 1442 cm^{-1} signified the presence of aromatic ring (C=C-C) bond vibrations, along with the 1041 cm^{-1} peak, which represented a strong C-O bond. These peaks collectively confirmed the presence of alcohols, carboxylic acid, alkanes, alkenes, aliphatic esters, carbonyl and aromatic compounds in DOEB. These findings led to the conclusion that the presence of polyphenols and flavonoids, substances known for exhibiting specific biological activities

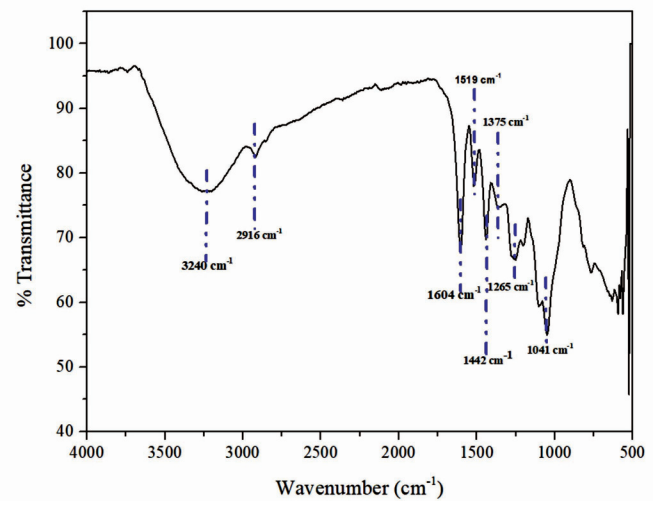

Figure 5: FT-IR spectral peaks obtained from DOEB

discussed in this study, could be confirmed through on the observed O-H (Devi and Battu, 2019; Batool *et al.*, 2020).

FTIR studies provided only preliminary screening of the compounds present in DOEB. Moreover, it is necessary to isolate the respective bioactive compounds that exhibit significant biological activities and identify their structures using advanced analytical methods, including mass spectroscopy and NMR spectroscopy.

CONCLUSION

This study provides a comprehensive understanding into the phytochemical composition and biological activities of the bark extract of *D. ovoideum*. The phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, terpenoids and steroids, known for their association with antioxidant, anti-inflammatory, anti-tyrosinase and antimicrobial activities. Notably, the high content of phenols and flavonoids contributed to the significant antioxidant activity of DOEB. The correlation between anti-inflammatory and radical scavenging activity centers on their combined ability of mitigate inflammation and oxidative stress, while the relationship between anti-tyrosinase activity and radical scavenging activity involves protecting against melanogenesis and oxidative damage, contributing to overall skin health. Polyphenols and steroids exhibit anti-inflammatory effects by inhibiting inflammatory enzymes and oxidative stress, while also demonstrating anti-tyrosinase activity by regulating melanin production and protecting against oxidative damage, collectively contributing to skin health and pigmentation balance. Additionally, DOEB demonstrated inhibitory effects against various bacterial strains *S. aureus*, *E. coli*, *K. pneumoniae* and the fungus *C. albicans*, suggesting its potential as an antimicrobial agent. DOEB emerges as a promising candidate for further investigation in the development of therapeutic agents due to its diverse range of phytochemicals and biological activities. Further investigation is necessary for the identification of bioactive compounds and the exploration of potential therapeutic applications.

Conflict of interest

The authors declare no conflict of interest.

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