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RESEARCH ARTICLE

GC-MS profiling and activity of *Diospyros melanoxylon* leaves against nosocomial pathogens

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Abstract

The rising incidence of microbial resistance and disorders associated with oxidative stress has propelled the quest for natural substances with therapeutic efficacy. The plant *Diospyros melanoxylon* (Roxb) known as tendu in Hindi & kendu in Odia, is indigenous to India (Odisha, Chhattisgarh, Jharkhand), Sri Lanka, Bangladesh and is a member of the Ebenaceae family. The current study is a chemometric analysis and antimicrobial study of *D. melanoxylon* leaf extract against some microorganisms responsible for hospital-acquired infections. Gas chromatography and Mass Spectroscopy (GC-MS) analysis was done to identify the phytocompounds present in the plant. The content of tannin, flavonoids and phenolics in *D. melanoxylon* was established using a colorimetric assay. Antioxidant activity was studied by assessing free radical scavenging assays. The *in vitro* antimicrobial assay was conducted using viable cell count and the turbidimetric method. The GC-MS analysis confirms the presence of various phytoconstituents like Epoxylanistan-11-ol, Phenol,2,4-bis(1,1 -dimethylethyl)-,Ur-12-2n-24-oic acid, α-amyrin & trimethylsilyl with α-amyrin being found to major constituent responsible for the pharmacological properties. The result indicated that ethyl acetate extract of *D. melanoxylon* leaves exhibited remarkable phenolic, flavonoid and tannin content. The plant extract has been effective against Gram-negative bacteria that cause hospital-acquired infections following liver transplantation, such as *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca and Pseudomonas aeruginosa.* These findings may be crucial in the development of new plant antibiotics against bacterial infections that predominantly happen following hospitalisation after liver transplantation. Further research should explore its mechanism of action, isolation and possible application in combination therapy.

Keywords: antimicrobial; antioxidant; *Diospyros melanoxylon*; GC-MS analysis; nosocomial pathogens

Abbreviation: ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, CFU: Colony-forming units, DPPH: 2,2-Diphenyl-1-picrylhydrazyl, GC-MS: Gas Chromatography Mass spectroscopy,H_2O_2:Hydrogen peroxide, LB: Luria Broth, MDR: Multi drug resistance, MH: Muller Hinton, MRSA: Methicillin-resistant Staphylococcus aureus, MS: Mass Spectroscopy, MSSA: Methicillin-sensitive Staphylococcus aureus, NIST: National institute of standard and Technology, PBS: Phosphate buffer solution, RT: Retention Time, TC: Tannin content, TFC: Total flavonoid content, TPC: Total phenolic content, UV: Ultra violet, WHO: World health organization

Introduction

Among the greatest medical advances of the past century, antibiotics are the most important class of drugs. Their contribution to the support of human society in combating microbes is not questionable and they have saved countless lives. Nevertheless, the appearance of multidrug-resistant (MDR) bacteria has become more widespread throughout the world during the twenty-first century and the risk of incurable infection has been an issue for decades (1-2). The problem of antibiotic resistance is one of the most significant issues worldwide. While drugs are enhancing their effectiveness, it has become harder to manage the newest list of infectious diseases. With the reduced effectiveness of antibiotics, the treatment of new lists of infectious diseases becomes more and more complex (3). Based on the World Health Organisation estimates that 700000 people will be affected by antimicrobial resistance and likely killing 10 million people each year and costing the world economy more than $100 trillion by 2050 (4). In addition, it is reported that transplant patients, especially liver transplant recipients, have high mortality and morbidity rates after the transplant due to microbial infection after surgery. Thus, infection control among liver transplant recipients is very challenging and requires immediate medical treatment. Since the devastating impact linked with gram-negative bacterial infections, modern medicine needs to look for new natural antimicrobial agents to fight against drug-resistant bacteria and the negative impact they produce on public health (5-7). The World Health Organization (WHO) has recently emphasised the importance of researching and developing novel, potent medicines to combat gram-

negative bacteria, since most of them have developed resistance (8). The increasing incidence of diseases and the discovery of new medicinal compounds from plants have led to the steady accumulation of scientific data on herbal drugs as valuable alternatives or complementary drugs for several diseases (9). It is stated that around 50000 plant species have been screened for their medicinal value and 80 % of the world's population uses these remedies in the treatment of many conditions, such as diabetes, ulcers, cancer, skin diseases, microbial infections and others (10). Numerous studies have demonstrated that a variety of bioactive substances, including phenolics, flavonoids, coumarins, alkaloids, tannins, terpenoids, essential oils, polypeptides, polyacetylenes and lectins, are present in medicinal plants. These bioactive compounds are the fundamental building blocks utilized for the synthesis of antibiotics, which are utilized in the treatment of various infectious diseases (11-12).

The plant *Diospyros melanoxylon* (Roxb), commonly known as tendu in Hindi, belongs to the family Ebenaceae, which is native to India (Odisha, Chhattisgarh, Jharkhand), Srilanka and Bangladesh. Indian traditional medicine has used *D. melanoxylon* leaves as a diuretic, styptic, laxative and carminative to treat night blindness and enhance vision. For bidis (cigarettes) manufacturing, coppiced leaves are highly valued (13-14). Particularly in the states of Odisha, Madhya Pradesh and Andhra Pradesh, the kendo (in Odia) leaf trade is a significant socioeconomic activity for Indian tribal people and a reliable source of income for the government (15 -16). The plant was reported to have different phytochemicals such as triterpenoids, flavonoids, tannins, phenolic, amino acid, protein, carbohydrate and steroid compounds found in the different extracts of the plants (17). The isolation of pentacyclic triterpenes like α- amyrin, β-amyrin, ursolic acid, oleanolic acid, dihydroxy triterpenic acid and β-sitosterol were done from the leaves of *D. melanoxylon* (13). Traditionally, the plant parts and whole plants have been extensively used as remedies to treat various diseases, as leaves are used as laxatives, carminatives and styptic diuretics, followed by bark, which is used to treat urinary discharge, flowers for skin disorders and inflammation (17). The reported pharmacological properties of the plant were found to be antidiabetic, analgesic, hepatoprotective and anti-inflammatory (18-19). The present research aimed to explore the presence of phytochemicals, *in vitro* antioxidant activity and in vitro antibacterial efficacy of *D. melanoxylon* leaf extract against the selected nosocomial pathogens.

Materials and Methods

Reagents and chemicals

2,2 -diphenyl-1-picrylhydrazide (DPPH) obtained from the Sigma Aldrich chemical, Hydrogen peroxide (H_2O_2), Griess reagent, ABTS reagent, Folin-Ciocalteu, folin denis reagent, sodium carbonate, sodium bicarbonate, aluminium chloride, trichloroacetic acid, sodium nitroprusside, potassium persulphate, gallic acid, ascorbic acid and quercetin were obtained from Merck Chemical. Luria broth, Nutrient Broth, Muller Hinton Broth and Muller Hinton (MH) agar were obtained from Hi Media, followed by Phosphate buffer saline (PBS) was obtained from Sigma Aldrich chemical. All the solvents used in the research work are analytical grade.

Collection and preparation of the plant material

Fresh leaves of *D. melanoxylon* were collected from the rural area near Bargarh District of Odisha, India, in the month of February. The same was authenticated by Dr. Gyanaranjan Mahallick, Associate Professor, Department of Botany, Centurion University of Technology and Management, School of Applied Sciences, Bhubaneswar, Odisha, with voucher no. CUTM/BOT/2024/03. Fresh leaves were washed with water and dried at room temperature in shade for nearly one week. A coarsely pulverised powder was prepared by drying the leaves.

Extract preparation

The initial weight of the plant material after air drying was 50 g, which was further macerated using n-hexane in a 4:1 ratio for 3 days, followed by evaporation of excess solvent using a rota evaporator. Then, the obtained plant residue was subjected to maceration with ethyl acetate extract. maceration with n-hexane, followed by extraction using ethyl acetate. These solvents were subjected to the 72 hr extraction treatment process before being filtered. Concentration of the resulting extracts to dryness at room temperature was carried out using a rotary evaporator. Out of all the extracts, the ethyl acetate extract was 8. % w/w in yield and stored at 2-8 °C for future studies (20).

Detection of plant materials by GC-MS analysis

The phytochemical composition of n-hexane and ethyl acetate leaves extract of *D melanoxylon* was examined by Thermo Trace 1300 gc and Thermo TSQ 800 Triple Quadrupole MS gas chromatograph interfaced with a mass spectrometer. The apparatus features a TG 5MS column (30m x 0.25mm, 0.25µm). The flow rate of the carrier gas, helium, was 1.0 mL/M per. The oven temperature was set to 60 °C for 15 min and then progressively raised to 280 °C for 3 min, while the injector was run at 250 °C. The constituents were identified by comparing them with those found in the GC-MS instrument's computer library (NIST and Wiley). The areas of each peak were compared to the chromatogram's overall area in order to calculate the relative percentages of the components that were found.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of n-hexane and ethyl acetate of *D. melanoxylon* was carried out to ascertain the presence of different secondary metabolites such as carbohydrates, alkaloid, protein, phenols, flavonoids, terpenoids, tannins, saponins and glycosides, which contribute to different pharmacological actions as per the researcher (21).

Determination of total phenolic, flavonoid and tannic acid content by the UV spectroscopy method

Total phenolic content determination

The total phenolic content (TPC) was determined by using a spectrophotometric method. 1 mL of Folin and Ciocalteu's phenol reagent was combined with 1 mL of the sample (concentration 1 mg/mL). The mixture was adjusted to 10 mL with distilled water after 3 min and 1 mL of saturated sodium carbonate solution was added. The absorbance at 725 nm was measured after the reaction was left in the dark for 90 min. The standard curve (20-100 µg/mL, Y=0.0192x+0.088, R2=0.9713) was constructed using gallic acid and the results were reported as µg of gallic acid equivalents/ mg of extract (GAES) (22).

Total flavonoid content determination

The amount of flavonoids in the extracts was determined using the spectrophotometric method (16). The 250 µL extract (1 mg/mL concentration) was mixed with 1.25 mL of distilled water and 75 µL

of 5 % $NaNO_2$ solution. 5 min later, 150 µL of a 10 % $AlCl_3$ solution was added. After 6 min, 500 µL of 1 M NaOH was added to 275 µL of distilled water to create the combination. The absorbance at 510 nm was measured after the solution had been well mixed. (±) quercetin was used to determine the standard curve (20-120 µg/mL, Y=0.4432x+0.337, R^2=0.9916) and the results were expressed as µg of (±) quercetin per milligram of extract (23).

Estimation of tannin content

The Folin-Denis assay was used to assess the tannin content of the extracts. The different extracts (50 µL) were mixed with double-distilled water to a final volume of 7.5 mL. The solution was then mixed with 1 mL of $Na_2CO_3$and 0.5 mL of Folin-Denis reagent. Double-distilled water was again used to achieve a volume of 10 mL. Absorbance was measured at 700 nm. A standard curve (20-120 µg/mL, Y=0.0069x+0.0091, R^2=0.9985) was plotted using tannic acid and the data were calculated as µg tannic acid equivalents per milligram of extract (24).

Determination of antioxidant capacity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

A modified version of a well-used technique was utilized to assess the DPPH radical scavenging ability of *D. melanoxylon* leaf extracts. Different quantities of ascorbic acid and extracts (12.5-200 µg/mL were taken. 3 mL of methanol, 1 mL of DPPH (0.15 mM in methanol) and 1mL of each sample were mixed and the mixture was let to kept at room temperature for 40 min in the dark. The mixture's absorbance at 517 nm was measured using a UV-Vis spectrophotometer. Three duplicates of the experiment were conducted (25).

The following formula was established to calculate the DPPH radical scavenging activity:

$$\frac{(Ac - As)}{Ac} \times 100 \qquad \text{Eqn. 1}$$

Where Ac is the absorbance of the control (Blank) and As is the absorbance of the different standard at various concentrations.

The concentration that scavenges 50 % of the DPPH is referred to as the IC_{50} value.

Reducing power assay

Antioxidant activity of leaf extracts from *D. melanoxylon* was evaluated using the reducing power test. Potassium ferricyanide (1 ml, concentration of 10 mg/mL), phosphate buffer (1 mL, 0.2 M, pH 6.6) and a variety of concentrations of different solvent extracts (1 mL) were mixed and incubated at 50 °C for 20 min. After adding 1 mL of trichloroacetic acid (100 mg/mL) to the mixture, it was centrifuged for 5 min at 8000 rpm. After mixing 1 mL of distilled water with 1 mL of supernatant and 0.1 mL of ferric chloride (1 mg/mL), the absorbance at 700 nm was measured (25).

Assay for hydrogen peroxide scavenging

With minor adjustments, the method outlined above was utilised to conduct the hydrogen peroxide radical scavenging assay. A 40 mM hydrogen peroxide was prepared by combining it with a 50 mM phosphate buffer at pH 7.4. To prepare the reaction mixture, 0.1 mL of ascorbic acid/extract was combined with 0.4 mL of the 50 mM phosphate buffer. 0.6 mL of the 40 mM H_2O_2 was then added to the mixture and it was allowed to stand for 10 min. The mixture was then read at 230 nm. Percentage inhibition was utilised to calculate and present the scavenging activity of the substances under investigation (25). The H_2Oscavenging ability was calculated using the formula:

$$\frac{(Ac - As)}{Ac} \times 100 \qquad \text{Eqn. 2}$$

Where Ac is the absorbance of the control (Blank) and As is the absorbance of the different standard at various concentrations

The ABTS radical scavenging activity

The process was used to evaluate the test materials' ABTS radical scavenging activity with minor modifications. 7 mM ABTS was added to a 2.45 mM potassium persulfate solution, which was then allowed to remain at room temperature in the dark for 12 hr. The solution was incubated for 15 min after 0.3 mL of extract solution and 2.7 mL of ABTS solution were combined for 45 sec for the photometric test. The mixture's absorbance was measured at 734 nm. The samples tested for radical scavenging activity were calculated and reported using percentage inhibition (26). The following formula was established to determine the ABTS radical scavenging capacity:

$$\frac{(Ac - As)}{Ac} \times 100 \qquad \text{Eqn. 3}$$

Where Ac is the absorbance of the control (Blank) and As is the absorbance of the different standard at various concentrations

Calculation of half maximal inhibitory concentration

It is the amount of antioxidant needed to scavenge 50 % of the free radicals, calculated by plotting the graph between the scavenging activity of the extract and its concentration.

***In vitro* antimicrobial study**

This study used five gram-negative bacteria as a test microorganism: *Acinetobacter baumanni* (MTCC 1425), *Escherichia coli* (MTCC 443), *Klebsiella oxytoca* (MTCC 3030), *Klebsiella pneumoniae* (MTCC 4031) and *Pseudomonas aeruginosa* (MTCC1688).

Viable cell count method

The viable cell count method was employed by the previously outlined protocol. Extract at a 100 µg/mL concentration was diluted 5000-fold and incubated for 2 hr at 37 °C with continuous shaking at 200 rpm. 100 µL of diluted culture from each reaction combination was applied to Luria Broth agar plates and thereafter incubated for 18 hr. Control was executed similarly, except addition of the extract. The total CFU on each plate was enumerated and compared to the control (26).

Next, the colony-forming units (CFU) on each plate were counted and compared to the control.

$$\% \text{ of inhibition} = 1 - (AS/AC \times 100) \qquad \text{Eqn. 4}$$

Where 'AS' is the absorbance of extraction at different concentrations

'AC' is the absorbance of the control

Broth-based turbidimetric assay

A turbidimetric broth-based assay technique, with slight modifications, was employed to measure the antibacterial activity of various *D. menaloxylon* extracts. A 100 µL inoculum, corresponding to 10^5 CFU, was agitated with 100 µL of plant extract at a concentration of 100 µg/mL at 37 °C for 2 hr. After incubation, 90 µL of the above mixture was added to 3 mL of LB and the resulting solution was incubated again for 18 hr at 37 °C. The

optical density of the mixture was measured at a wavelength of 600 nm. Except for the extract, the control was also prepared similarly. Three independent calculations were performed to calculate the percentage of inhibition for the treated samples (27).

Inhibition percentage = 1-((AS/AC) ×100 Eqn. 5

Where 'AS' is the absorbance of extraction at different concentrations

'AC' is the absorbance of the control

Results and Discussion

GC-MS analysis

The result pertaining to GC-MS analysis leads to the identification of several compounds from the n-hexane and ethyl acetate extract of the DM leaves. These compounds were identified through mass spectroscopy attached to GC. A chromatogram of n-hexane extract & ethyl acetate extract of *D. melanoxylon* leaves of different phytocompounds was obtained from GC-MS analysis (Fig. 1A & B). Chemical mixtures are separated by the instrument's GC component, while molecular-level component identification is accomplished by the MS component. The identification of the substance was based on comparing its mass, retention index and retention time spectra with those in the NIST library. Noteworthy is the fact that the primary chemical was identified with good separation. Epoxylanistan-11-ol (RT 21.88), Phenol, 2,4-bis(1,1-dimethylethyl)- (RT 9.60), Urs-12-2n-24-oic acid, oxomethylester (RT 17.19), α-amyrin, trimethylsilyl ether (RT 11.25), α-amyrin (RT 17.11) were the main active compound found in n-hexane and ethyl acetate extract. It is commonly recognized that α-amyrin and β-amyrin are a naturally occurring triterpene combination. Previous research has documented its pharmacological potential, which includes hepatoprotective, anticancer, anti-inflammatory and antioxidant effects. According to reports, α-amyrin may have antibacterial properties against the bacterium (28, 29). The reference strains of methicillin-resistant and methicillin-sensitive *S. aureus* (MRSA and MSSA, respectively) showed markedly decreased biomass and metabolic activity when α-amyrin (AM), which is generated from ursane, was used in a prior study (29, 30). Urs-12-2n-24-oic acid, a derivative of ursolic acid, is known to possess antioxidant, antibacterial and anti-inflammatory qualities (31). It has been reported that, α-amyrin has also been active against different gram-negative microorganisms (32). The various components present in the leaf of DM that were detected by the GC-MS are shown in Table 1 and 2.

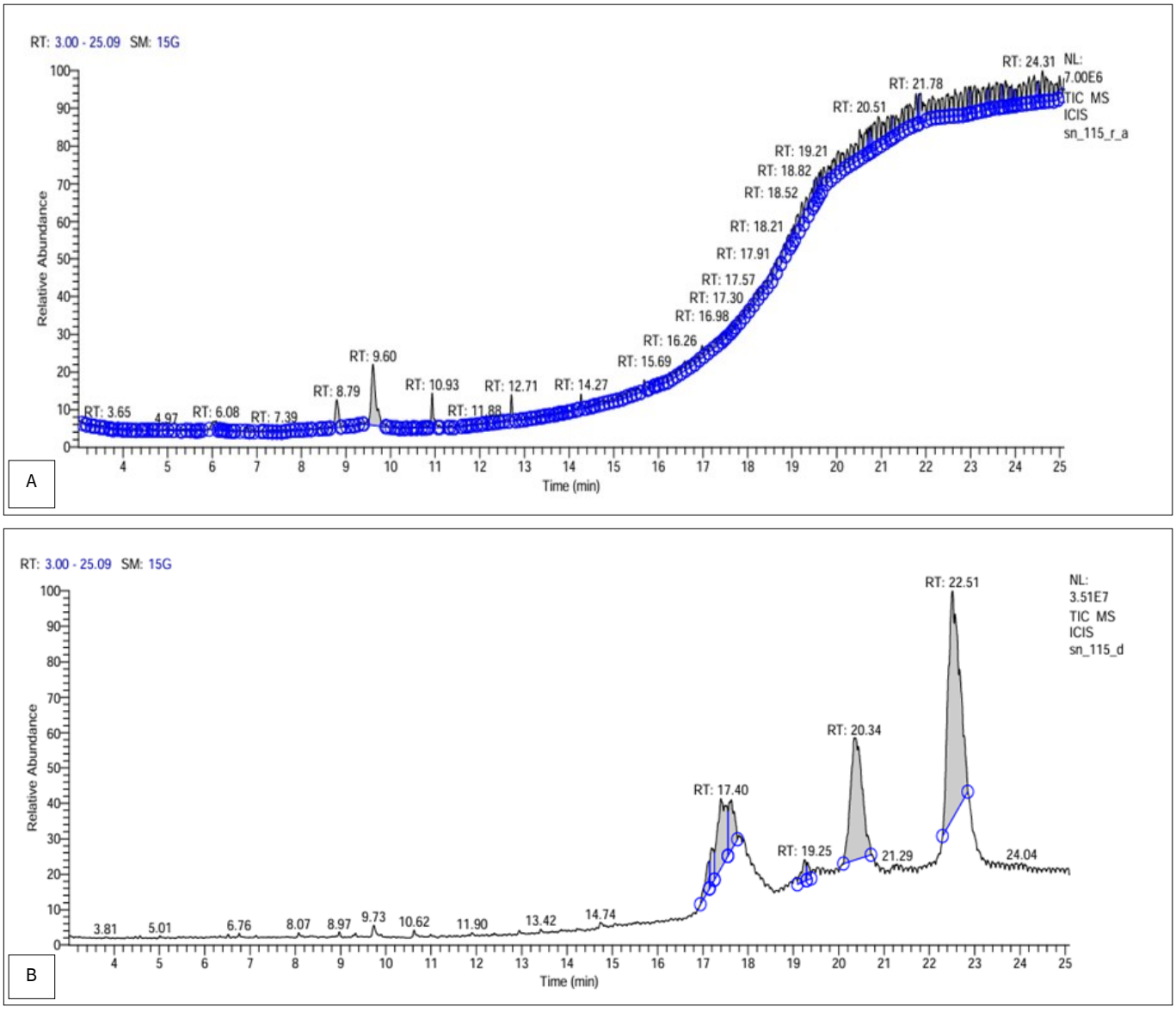

Fig. 1A & B. GC-MS chromatogram of n-hexane and ethyl acetate extract of *D. melanoxylon* leaves.

Table 1. Compounds identified in n-hexane extract of DM through GC-MS analysis

Sl no.	RT	Name of the compound	Molecular formula	% Area	Molecular weight	Uses	Reference
1	8.79	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	1.69	519.07	Immunomodulatory, antifungal, antimicrobial	33
2	9.60	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	5.16	206.32	Antibacterial, antifungal, anti-inflammatory	34
3	20.31	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	1.00	563	Larvicidal, Anti-inflammatory, antibacterial, antifungal, anticancer	35
4	20.67	9,12,15-Octadecatrienoic acid,	$C_{27}H_{52}O_4Si_2$	1.34		Anti-infllamtoty, antiparasite	36
	20.85	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	1.53	436.6	Antioxidant, antimicrobial, anticancer	37
5	20.93	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	1.40	488.88	Antimicrobial, antoxidant, anti-inflammatory, anticancer	37
6	21.88	7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	2.02	502.8	Anti-inflammatory, antimicrobial	38
7	20.03	Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris [(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	$C_{36}H_{69}NO_6Si_3$	1.18	696.2	Aantibacterial	39
8	21.63	9,12,15-Octadecatrienoic acid, 2,3-bis [(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)	$C_{27}H_{52}O_4Si_2$	1.10	496.9	Anti-inflammatory, hepatoprotective	40
9	24.42	Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R)-	$C_{27}H_{40}O_4$	1.36	428.56	Antioxidant, antimicrobial, antiinflammatory	41

Table 2. Compounds identified in ethyl acetate extract of DM through GC-MS analysis

Sl no.	RT	Name of the compound	Molecular formula	% Area	Molecular weight	Compound Nature	Uses	Reference
1	17.11	à-amyrin	$C_{30}H_{50}O$	2.40	426.7	Pentacyclic terpenes	Antimicrobial, anti-inflammatory, hepatoprotective	42
2	17.19	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	$C_{31}H_{48}O_3$	2.57	468.7	Terpenes	Antioxidant, anticancer, anti inflammation	43
3	17.40	à-Amyrin, trimethylsilyl ether	$C_{33}H_{58}OSi$	11.25	489.89	Terpenes	Anti-inflammatory	42
4	17.58	3-hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5, 6,7,8-octahydronaphthalen-2-yl ester	$C_{17}H_{26}O_3$	5.36	278.4	Ester derivative	Anti-inflammatory, antibacterial	43
5	19.25	Lup-20(29)-en-3-ol, acetate, (3á)-	$C_{32}H_{52}O_2$	1.90	468.75	Pentacyclic triterpenoids	Antioxidant, anticancer, antiinflmmatory, antimicrobial	43
6	19.31	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	0.92	436.6	Steroidal	Antiinflammatory, antidiabetic, antioxidant	43
7	20.34	4,4,6a,6b,8a,11,11,14b-Octamethyl 1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H	$C_{30}H_{48}O$	26.91	424.7	triterpenoids	Antioxidant, antibacterial	43

Preliminary phytochemical analysis

Phytochemical analysis of extracts of *D. melanoxylon* was performed to ascertain the presence secondary metabolites, which revealed the presence of carbohydrates, alkaloids, steroids, proteins, terpenoids, glycosides, tannins, phenolic and flavonoids as major phytoconstituents of this plant as shown on the Table 3 below. As per the literature it has been found the presence of these phytoconstituents may contribute towards the antibacterial activity. These plant secondary metabolites disrupt the cellular function of bacteria (21).

Determination of total phenolic, flavonoid and tannic acid content by UV spectroscopy method

D. melanoxylon leaf extract showed significant tannic acid, flavonoid and phenolic contents (Table 4). The highest TPC, TFC & TC content was obtained in the ethyl acetate extract of *D. melanoxylon* leaves. The various molecular and biological factors that contribute to *D. melanoxylon* leaf extract's antibacterial efficacy are mostly related to their hydroxyl groups and electron delocalisation, which interact with the proteins, organelles and cell membranes of microorganisms. These interactions have the potential to harm the

Table 3. Phytochemical analysis of different extract of *D. melanoxylon* leave

Test			N-hexane extract	Ethyl acetate extract
		Interference	Significance	Significance
Alkaloids	Drangendroff's test	Orange precipitate	+	+
	Mayer's test	Cream precipitate	+	+
	Wagner's test	Yellow precipitate	+	+
Anthraquinone gylcosides	Borntranger's test	Pink colour	-	-
Carbohydrates	Benedicts' s test	Red precipitate	+	+
	Felhinhg's test	Brick red precipitate	+	+
	Molis's test	Violet ring	+	+
	Selwinoff's test	Pink colour	+	+
Flavonoids	Shinoda test	Pink colour	+	++
Phenolics	1 % Ferric chloride	Black blue colour	+	++
Saponins	Froth test	Frothing	++	+
	Foaming test	Foaming	+	+
Tannins	5 % Ferric Chloride	Yellow colour	+	++
Terpenoids	Salkowski's test	Golden yellow ring at junction	+	++

(Note: - sign indicates absence and + sign indicates presence)

Table 4. Determination of phytochemical constituents through UV method

Sample	Total flavonoid content	Total phenolic content	Tannin content
Ethyl acetate	31.34±3.1	72.31±3.4	30.34±3.8
n-hexane	27.32±2.3	68.34±3.3	25.34±3.24

Means ± standard error (n = 3)

cell membrane and decrease the effectiveness of metabolic pathways. Additionally, polyphenols have a unique capacity to combine with metal ions to form complexes, which enhances their antibacterial activity. Cell death or damage to the cell membrane may result from the interaction between DMs and the microbial cell (44). Additionally, researchers have demonstrated the antibacterial and antioxidant activity of flavonoids by analysing their chemical structure. From the results, it has been seen that *D. melanoxylon* leaves contain a presence of polyphenols, which may contribute towards the antimicrobial activity.

DPPH radical scavenging activity

In this study, the ethyl acetate and n-hexane extracts of *D. melanoxylon* were analysed for antioxidant activity using the DPPH radical scavenging assay. The percentages of DPPH radical scavenging activity of ascorbic acid were found to be 101.32 at higher concentrations. DPPH radicals were scavenged by the extract in a dose-dependent manner. n-Hexane had the lowest radical scavenging activity, whereas the ethyl acetate extract had the highest DPPH radical scavenging activity. This finding reflected the anti-oxidative activity of the ethyl acetate extract. Given that the DPPH assay is a reduction mechanism-based assay, the ethyl acetate extract likely had a greater quantity of DPPH reductions than n-hexane (Table 5). The findings demonstrated that *D. melanoxylon* leaf extract in ethyl acetate had better free radical scavenging activity and contributed towards the antioxidant activity. The organic substances that are mostly derived from natural sources and play a major role in the body's defence mechanism are antioxidants, which include polyphenols, vitamins and carotenoids. Antioxidants can indirectly inhibit bacterial growth due to their radical scavenging activity and oxidative stress reduction (44, 45). As *D. melanoxylon* has significant antioxidant capacity, it could be to suitable agent to have antimicrobial efficacy.

H_2O_2 scavenging activity

Table 6 represents the percentages of H_2O_2 scavenging activity. A highly reactive oxygen-centred radical known as a hydroxyl radical is created when different hydrogen peroxides react with transition metal ions. It targets proteins. DNA, membrane-bound polyunsaturated fatty acids and most biological molecules (46, 47). Ethyl acetate extract exhibited the greatest scavenging activity of H_2O_2, whereas n-hexane exhibited moderate scavenging activity. When the concentration was raised, the scavenging activity of both extracts was enhanced.

Table 5. DPPH Scavenging assay (% inhibition)

Concentration	% of inhibition ascorbic acid	% of inhibition n-hexane extract	% of inhibition Ethyl acetate extract
12.5	59.23±2.32	19.34±0.73	54.57±2.43
25	66.79±2.63	25.34±1.23	58.37±2.32
50	74.38±2.72	53.17±2.34	62.45±2.34
100	87.95±2.39	67.32±2.34	66.54±3.28
200	98.32±3.17	73.13±3.4	68.35±2.72
IC_{50}	8.5	47.1	12.5

Values were in mean ± standard deviation, n=3

Table 6. Hydrogen peroxide assay (% inhibition)

Concentration	% of inhibition ascorbic acid	% of inhibition n-hexane extract	% of inhibition Ethyl acetate extract
12.5	57.38±2.32	14.35±1.34	42.45±2.1
25	63.39±3.23	17.39±1.72	46.22±2.45
50	73.24±3.4	27.35±1.51	51.45±2.6
100	85.32±3.2	34.39±2.34	56.37±3.4
200	91.38±3.4	37.84±2.27	62.21±2.9
IC_{50}	10.9	101.2	43.07

Values were in mean ± standard deviation, n=3

Reducing power assay

Table 7 represents the percentages of reducing power capacity of plant extracts and the standard. Depending on each compound's reducing capability, the test solution's yellow colour in this assay shifts to different colours of green and blue. The $Fe3^+$/ferricyanide complex is reduced to the ferrous form when reducers are present. The presence of a reductant, which has been demonstrated to have antioxidant effects by donating a hydrogen atom to break the chain of free radicals, is typically linked to the reducing capabilities (48). Ethyl acetate extract exhibited the greatest activity, whereas n -hexane exhibited moderate scavenging activity. When the concentration was raised, the scavenging activity of both extracts was enhanced; also it is equivalent to standard ascorbic acid. The mechanism of reducing the power of extracts is similar to previously reported work.

ABTS radical scavenging activity

Antioxidants play a vital role in defence and advancing health in living organisms against the free radicals. Antioxidant potential of plants is due to oxidation reaction of phenolic acid flavonoid compounds present in them that help in hydrogen donors by losing electrons, reducing agents and singlet oxygen quenchers. Our research shows the presence of flavonoids and phenolic compounds in *D. melanoxylon.* The ABTS+ cation-free radical scavenging assay is a common method to assess a plant's antioxidant capacity. The ethyl acetate extract of *D. melanoxylon* exhibited ABTS free radical scavenging capacity (93.6) as compared to standard ascorbic acid (Table 8) (21).

Table 7. Reducing power assay (% inhibition)

Concentration	% of inhibition ascorbic acid	% of inhibition n-hexane extract	% of inhibition Ethyl acetate extract
12.5	72.35 ±3.2	17.34 ± 1.56	34.17 ± 1.77
25	75.64 ± 3.3	22.39 ± 1.7	36.74 ± 2.7
50	81.34 ± 3.3	25.37 ± 2.4	42.1 ± 2.27
100	88.57 ± 3.68	27.29 ± 2.8	46.22 ± 2.7
200	96.32 ± 4.4	32.05 ± 1.5	52.74 ± 2.5
IC_{50}	8.6	64.9	52.22

Values were in mean ± standard deviation, n=3

Table 8. ABTS assay (% on inhibition)

Concentration	% of inhibition ascorbic acid	% of inhibition n-hexane extract	% of inhibition Ethyl acetate extract
12.5	32.4 ± 1.2	8.4 ± 1.0	15.6 ±1.4
25	48.9 ± 1.5	15.2 ± 1.3	23.8 ± 1.8
50	68.7 ± 2.0	26.7 ± 1.8	35.4 ± 2.1
100	85.3 ± 2.5	39.4 ± 2.1	52.7 ± 2.3
200	94.6 ± 1.8	55.6 ± 2.4	71.2 ± 2.6
IC_{50}	26.3	165.4	93.6

In vitro antimicrobial assay of ethyl acetate extract of *D. melanoxylon* using broth-based turbidimetric method

Since the broth-based turbidimetric assay is a simpler and more reproducible method than the agar-based assay, the latter was used for the assessment of antibacterial activity of ethyl acetate leaf extract of *D. melanoxylon*. From the GC-MS data and quantitative phytochemical estimation, it has been found that ethyl acetate extract of *D. melanoxylon* leaves has greater value compared to n-hexane extract in terms of phenolic, flavonoid and tannin contents. From the literature, it was found that flavonoids are a class of phytochemicals with potentially beneficial properties, including direct antibacterial effects, enhancement of antibiotic efficacy and inhibition of bacterial pathogenicity (49). Phenolic compounds with a flexible scaffold that also have powerful antibacterial properties via acting on the bacterial cell, affecting cell structure and shape, or disrupting bacterial metabolism (50). Hence, ethyl acetate extract will be a better candidate for the estimation of antimicrobial activity. They performed at a concentration ranging from 25-400 µg/mL. Sterile distilled water (control) did not show any inhibition against the microorganism. The microbial growth of these gram-negative bacteria, namely *A. baumanii, E. coli*, *K. pneumoniae* and *P. aurigonosae,* was found to be inhibited by *D. melanoxylon*. According to data shown in Fig. 2, the growth of gram-negative bacteria is found to be resistant. It exhibited dose-dependent antibacterial activity against all tested bacteria. Interestingly, *D. melanoxylon* leaf extract exhibited intense antibacterial activity against gram-negative bacteria and suppressed microorganism growth.

In vitro antimicrobial assays of the ethyl acetate extract of *D. melanoxylon*

The ethyl acetate leaf extract of *D. melanoxylon* was added to nutrient agar plates with gram-negative bacteria in different concentrations (25-400 µg/mL). Based on the results, as shown in Fig. 2, the plant showed promising antibacterial activity against all gram-negative bacteria tested. The antibacterial activity against *E. coli* and *A. baumannii* was significantly higher. The microbial strains tested showed a dose-dependent effect (Fig. 2). Therefore, it was found that *D. melanoxylon* has potential in the treatment of bacterial infections. Gram-negative pathogen-induced infections were found to be much more difficult to treat because of their high intrinsic drug resistance. The existence of an outer membrane that serves as a permeability barrier causes the development of this intrinsic drug resistance. *D. melanoxylon* showed dose-dependent antibacterial efficacy against every test microorganism.

Conclusion

The study sought to compare the total phenolic content, flavonoid, tannin content, antioxidant and antimicrobial activities of *D. melanoxylon* leaves extract. This study showed that *D. melanoxylon* extract showed significant phenolic, flavonoid and tannin content, followed by antioxidant activity. Antimicrobial efficacy of *D. melanoxylon* against gram-negative bacteria positions it as a promising candidate for antimicrobial therapy, particularly in light of the growing need for alternative therapies against resistant strains of bacteria. α-amyrin among the various compounds detected through GC-MS analyses and from the literature, it was found that it may be a potential candidate for antimicrobial. It is thought to be a potential candidate for antimicrobial activity. Hence this research work will aid in paving the path to examine the antibacterial potential of D melanoxylon leaves against the nosocomial pathogens. Future studies should focus on isolation, elucidating the specific mechanisms of action of α-amyrin and exploring its potential for use in combination therapies.

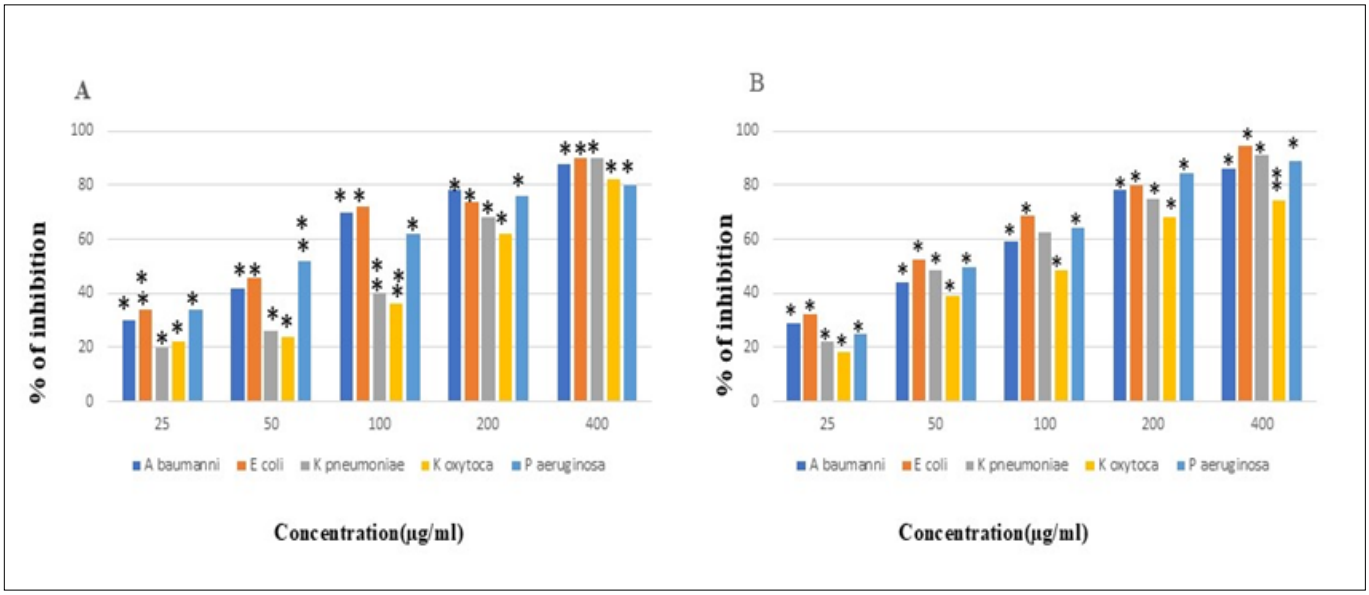

Fig. 2. *In vitro* antimicrobial assay of *D. melanoxylon* extract (Viable cell count method and turbidimetry method).

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Authors' contributions

SB and BK were responsible for the study design. SB and CD performed the phytochemical analysis. AN, AKD and SB conducted the antimicrobial work. GG and GR handled the editorial work. All authors reviewed and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors have no conflict of interest to declare.

Ethical issues: None

References

1. Huy TXN. Overcoming *Klebsiella pneumoniae* antibiotic resistance: new insights into mechanisms and drug discovery. Beni-Suef Univ J Basic Appl Sci. 2024;13(1):13. https://doi.org/10.1186/s43088-024-00470-4
2. Cook GD, Stasulli NM. Employing synthetic biology to expand antibiotic discovery. SLAS Technol. 2024;29(2):100120. https://doi.org/10.1016/j.slast.2024.100120
3. Aggarwal R, Mahajan P, Pandiya S, Bajaj A, Verma SK, Yadav P, et al. Antibiotic resistance: a global crisis, problems and solutions. Crit Rev Microbiol. 2024;50(5):896-921. https://doi.org/10.1080/1040841X.2024.2313024
4. Mularoni A, Cona A, Campanella M, Barbera F, Medaglia AA, Cervo A, et al. Donor-derived carbapenem-resistant gram-negative bacterial infections in solid organ transplant recipients: active surveillance enhances recipient safety. Am J Transplant. 2024;24(6):1046-56. https://doi.org/10.1016/j.ajt.2024.02.005
5. Bonazzetti C, Rinaldi M, Cosentino F, Gatti M, Freire MP, Mularoni A, et al. Survey on the approach to antibiotic prophylaxis in liver and kidney transplant recipients colonized with “difficult to treat” gram-negative bacteria. Transpl Infect Dis. 2024;26(2):e14238. https://doi.org/10.1111/tid.14238
6. Yang W, Li J, Yao Z, Li M. A review on the alternatives to antibiotics and the treatment of antibiotic pollution: current development and future prospects. Sci Total Environ. 2024;926:171757. https://doi.org/10.1016/j.scitotenv.2024.171757
7. Abdulrahman MD. Antioxidant, alpha glucosidase and antibacterial evaluation of *Syzygium mytifolium* (Roxb.) Walp. Plant Sci Today. 2021;8(2):410-15. https://doi.org/10.14719/pst.2021.8.2.1113
8. World Health Organization. WHO bacterial priority pathogens list, 2024: bacterial pathogens of public health importance, to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva: World Health Organization; 2024.
9. Singh R, Prasad KK, Siddiqui MW, Prasad K. Medicinal plants in preventive and curative roles for various ailments. In: Plant Secondary Metabolites. Vol. 1. Apple Academic Press; 2017:83-120. https://doi.org/10.1201/9781315366326-11
10. Ferdes M. Antimicrobial compounds from plants. In: Fighting Antimicrobial Resistance. Zagreb: IAPC-OBP; 2018:243-71. https://doi.org/10.5599/obp.15.15
11. Ndomou SCH, Mube HK. The use of plants as phytobiotics: a new challenge. In: Phytochemicals in Agriculture and Food. IntechOpen; 2023.
12. Hilal B, Khan MM, Fariduddin Q. Recent advancements in deciphering the therapeutic properties of plant secondary metabolites: phenolics, terpenes and alkaloids. Plant Physiol Biochem. 2024;211:108674. https://doi.org/10.1016/j.plaphy.2024.108674
13. Mallavadhani UV, Panda AK, Rao YR. *Diospyros melanoxylon* leaves: a rich source of pentacyclic triterpenes. Pharm Biol. 2001;39(1):20-4. https://doi.org/10.1076/phbi.39.1.20.5941
14. Patel B, Nayak B, Behera S, Parida S. Exploring nutritional and value -added products of *Diospyros melanoxylon* (Roxb.) fruits. J Adv Zool. 2024;45(2).
15. Qureshi FQF, Reddy VVRV. Systematic review on phytochemical and pharmacological aspects of *Diospyros melanoxylon* Roxb. J Pharmacol Pharm Res. 2025;2(1):24. https://doi.org/10.5455/JPPR.20250117044642
16. Rathore K, Singh VK, Jain P, Rao SP, Ahmed Z, Singh VD. In-vitro and in-vivo antiadipogenic, hypolipidemic and antidiabetic activity of *Diospyros melanoxylon* (Roxb). J Ethnopharmacol. 2014;155(2):1171 -6. https://doi.org/10.1016/j.jep.2014.06.050
17. Saraf PN, Srivastava J, Munoz F, Charles B, Samal P, Quamar MF. Ecological niche modelling to project past, current and future distributional shift of black ebony tree *Diospyros melanoxylon* Roxb. in India. Nord J Bot. 2024;2024(6):e04266. https://doi.org/10.1111/njb.04266
18. Patel J, Reddy V, Kumar GS. Evaluation of hepatoprotective activity of ethanolic extract of *Diospyros melanoxylon* (Roxb) leaves against CCl4 induced hepatotoxicity in albino rats. Res J Pharm Technol. 2015;8(5):571-4. https://doi.org/10.5958/0974-360X.2015.00095.5
19. Al Rashid MH, Majumder S, Mandal V, Mandal SC, Thandavarayan RA. In search of suitable extraction technique for large scale commercial production of bioactive fraction for the treatment of diabetes: the case *Diospyros melanoxylon* Roxb. J Tradit Complement Med. 2019;9(2):106-18. https://doi.org/10.1016/j.jtcme.2017.11.003
20. Dash S, Sahoo N, Pattnaik G, Das C, Pattanaik S, Ghosh G, et al. *In vitro* and in-silico anti-diabetic evaluation of the combination of *Annona squamosa* Linn. leaf extract and oleanolic acid. Curr Bioact Compd. 2024;20(10):E150224227005. https://doi.org/10.2174/0115734072294929240206060527
21. Rao H, Rao I, Saeed L, Aati HY, Aati S, Zeeshan M. Phytochemical analysis and bioactivity assessment of five medicinal plants from Pakistan: exploring polyphenol contents, antioxidant potential and antibacterial activities. Saudi J Biol Sci. 2023;30(10):103783. https://doi.org/10.1016/j.sjbs.2023.103783
22. Bolade OP, Williams AB, Benson NU. Dataset on analytical characterization of bioactive components from *Azadirachta indica*, *Canna indica*, *Mangifera indica* and *Moringa oleifera* leaf extracts and their applications in nanoparticles biosynthesis. Data Brief. 2021;38:107407. https://doi.org/10.1016/j.dib.2021.107407
23. Guemari F, Laouini SE, Rebiai A, Bouafia A, Meneceur S, Tliba A, et al. UV-visible spectroscopic technique-data mining tool as a reliable, fast and cost-effective method for prediction of total polyphenol contents: validation in a bunch of medicinal plant extracts. Appl Sci. 2022;12(19):9430. https://doi.org/10.3390/app12199430
24. Dash P, Ghosh G. Proteolytic and antioxidant activity of protein fractions of seeds of *Cucurbita moschata*. Food Biosci. 2017;18:1-8. https://doi.org/10.1016/j.fbio.2016.12.004
25. Fawwaz M, Pratama M, Musafira M, Wahab I, Iriani R, Aminah A, et al. Evaluation of antioxidant activity of *Vernonia amygdalina* leaves and its flavonoid-phenolic content. Indones J Pharm Sci Technol. 2023;10(2):104-10. https://doi.org/10.24198/ijpst.v10i2.41617

26. Othman M, San Loh H, Wiart C, Khoo TJ, Lim KH, Ting KN. Optimal methods for evaluating antimicrobial activities from plant extracts. J Microbiol Methods. 2011;84(2):161-6. https://doi.org/10.1016/j.mimet.2010.11.008

27. Okoye NN, Ajaghaku DL, Okeke HN, Ilodigwe EE, Nworu CS, Okoye FBC. Beta-amyrin and alpha-amyrin acetate isolated from the stem bark of *Alstonia boonei* display profound anti-inflammatory activity. Pharm Biol. 2014;52(11):1478-86. https://doi.org/10.3109/13880209.2014.898078

28. Alsaedi HK, Alwan NA, Al-Masoudi EA. Physiological and biochemical effect of α-amyrin: a review. J Med Life Sci. 2024;6(3):443-52. https://doi.org/10.21608/jmals.2024.383360

29. da Silva Júnior WF, Pinheiro JGDO, De Menezes DLB, e Silva NEDS, De Almeida PDO, Lima ES, et al. Development, physicochemical characterization and *in vitro* anti-inflammatory activity of solid dispersions of α, β-amyrin isolated from *Protium* oilresin. Molecules. 2017;22(9):1512. https://doi.org/10.3390/molecules22091512

30. Galal SM, El Kiki SM, Elgazzar EM. The potential therapeutic approach of ursodeoxycholic acid as a potent activator of ACE-2 on cerebral disorders induced by γ-irradiation in rats. Cell Biochem Funct. 2024;42(8):e70024. https://doi.org/10.1002/cbf.70024

31. Chung PY, Navaratnam P, Chung LY. Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against *Staphylococcus aureus* strains. Ann Clin Microbiol Antimicrob. 2011;10(1):25. https://doi.org/10.1186/1476-0711-10-25

32. Kasim Mohamed K, Fathima Banu R, Anand Kumar V, Sundaram L, Thyagarajan SP. GC-MS and surface characteristics of polyvinyl siloxane-an *in vitro* analysis. J Chromatogr Sci. 2022;60(2):111-16. https://doi.org/10.1093/chromsci/bmab054

33. Mostofa MG, Reza AA, Khan Z, Tsukahara T, Alam AK, Sadik MG. The apoptosis-inducing antiproliferative activity and quantitative phytochemical profiling of polyphenol-rich part of *Leea aequata* L. leaves. 2021. https://doi.org/10.21203/rs.3.rs-830741/v1

34. Gurunathan A, Senguttuvan J, Paulsamy S. Evaluation of mosquito repellent activity of isolated oleic acid, eicosyl ester from *Thalictrum javanicum*. Indian J Pharm Sci. 2016;78(1):103. https://doi.org/10.4103/0250-474X.180259

35. Parvathi K, Kandeepan C, Sabitha M, Senthilkumar N, Ramya S, Boopathi NM, et al. In-silico absorption, distribution, metabolism, elimination and toxicity profile of 9,12,15-octadecatrienoic acid (ODA) from *Moringa oleifera*. J Drug Deliv Ther. 2022;12(2-S):142-50. https://doi.org/10.22270/jddt.v12i2-S.5289

36. Malathi K, Anbarasu A, Ramaiah S. Ethyl iso-allocholate from a medicinal rice Karungkavuni inhibits dihydropteroate synthase in *Escherichia coli*: a molecular docking and dynamics study. Indian J Pharm Sci. 2017;78(6):780-8. https://doi.org/10.4172/pharmaceutical-sciences.1000184

37. Alqahtani SS, Makeen HA, Menachery SJ, Moni SS. Documentation of bioactive principles of the flower from *Caralluma retrospiciens* (Ehrenb) and *in vitro* antibacterial activity-Part B. Arab J Chem. 2020;13(10):7370-7. https://doi.org/10.1016/j.arabjc.2020.07.023

38. Naz S, Alam S, Ahmed W, Khan SM, Qayyum A, Sabir M, et al. Therapeutic potential of selected medicinal plant extracts against multi-drug resistant *Salmonella enterica* serovar typhi. Saudi J Biol Sci. 2022;29(2):941-54. https://doi.org/10.1016/j.sjbs.2021.10.008

39. Al Bratty M, Makeen HA, Alhazmi HA, Syame SM, Abdalla AN, Homeida HE, et al. Phytochemical, cytotoxic and antimicrobial evaluation of the fruits of miswak plant *Salvadora persica* L. J Chem. 2020;2020(1):4521951. https://doi.org/10.1155/2020/4521951

40. Baburaj R, Sandur RV, Das K. Comparative *in vitro* anti-inflammatory study on alpha amyrin isolated from bark extracts of *Ficus benghalensis* Linn and *Alstonia boonei* De Wild. RGUHS J Pharm Sci. 2022;12(4). https://doi.org/10.26463/rjps.12_4_3

41. Oh KK, Adnan M, Cho DH. Network pharmacology-based study to uncover potential pharmacological mechanisms of Korean thistle (*Cirsium japonicum* var. *maackii* (Maxim.) Matsum.) flower against cancer. Molecules. 2021;26(19):5904. https://doi.org/10.3390/molecules26195904

42. Abdelhamid MS, Kondratenko EI, Lomteva NA. GC-MS analysis of phytocomponents in the ethanolic extract of *Nelumbo nucifera* seeds from Russia. J Appl Pharm Sci. 2015;5(4):115-18. https://doi.org/10.7324/JAPS.2015.50419

43. Akwu NA, Naidoo Y, Singh M, Nundkumar N, Lin J. Phytochemical screening, *in vitro* evaluation of the antimicrobial, antioxidant and cytotoxicity potentials of *Grewia lasiocarpa* E. Mey. ex Harv. S Afr J Bot. 2019;123:180-92. https://doi.org/10.1016/j.sajb.2019.03.004

44. Bruno-Bárcena JM, Azcárate-Peril MA, Hassan HM. Role of antioxidant enzymes in bacterial resistance to organic acids. Appl Environ Microbiol. 2010;76(9):2747-53. https://doi.org/10.1128/AEM.02718-09

45. Bolade OP, Williams AB, Benson NU. Dataset on analytical characterization of bioactive components from *Azadirachta indica*, *Canna indica*, *Mangifera indica* and *Moringa oleifera* leaf extracts and their applications in nanoparticles biosynthesis. Data Brief. 2021;38:107407. https://doi.org/10.1016/j.dib.2021.107407

46. Tian C, Liu X, Chang Y, Wang R, Lv T, Cui C, et al. Investigation of the anti-inflammatory and antioxidant activities of luteolin, kaempferol, apigenin and quercetin. S Afr J Bot. 2021;137:257-64. https://doi.org/10.1016/j.sajb.2020.10.022

47. Gulcin İ. Antioxidants: a comprehensive review. Arch Toxicol. 2025;1-105.

48. Gulcin İ, Alwasel SH. Fe3+ reducing power as the most common assay for understanding the biological functions of antioxidants. Processes. 2025;13(5):1296. https://doi.org/10.3390/pr13051296

49. Cushnie TT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents. 2011;38(2):99-107. https://doi.org/10.1016/j.ijantimicag.2011.02.014

50. Lobiuc A, Pavăl NE, Mangalagiu II, Gheorghiță R, Teliban GC, Amăriucăi-Mantu D, et al. Future antimicrobials: natural and functionalized phenolics. Molecules. 2023;28(3):1114. https://doi.org/10.3390/molecules28031114

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