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ANTIMICROBIAL, PHYTOCHEMICAL AND TOXICOLOGICAL PROFILE OF ESSENTIAL OIL EXTRACTED FROM *PANDANUS ODORIFER* WITH AN AVAILABLE SIMULATION STUDY

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ABSTRACT

Plant-derived essential oils are natural sources of antimicrobial and pharmaceutical agents besides their broad spectrum of use in the cosmetic, food, and perfume industries. The essential oil from the flowers of *P. odorifer* was extracted using the hydro distillation method. The major compound from the essential oil was identified by high-resolution mass spectroscopy (HRMS) analysis the identified phytochemicals include p-benzoquinone, 2-phenethyl alcohol, p-cymene, 2-phenethyl methyl either alpha-Terpineol, Psoralen, Isoplumbagin, Genipin, Artemidiol, Pinocembrin, (-)-Glycinol and, Iprobenfos. Two of its major phytochemicals,2-phenethyl methyl ether (PEME) and alpha-terpineol identified through HRMS were procured from Sigma-Aldrich. The antimicrobial properties of the PEME, essential oil, and terpineol was determined using agar well diffusion and micro broth dilution method against four clinically isolated multidrug-resistant (MDR) pathogenic bacteria viz. *Staphylococcus aureus*, *Streptococcus pyogenes*, *Acinetobacter baumanni*, and *Pseudomonas aeruginosa*. PEME and alpha-terpineol exhibited significant antibacterial activity against all the isolated pathogenic MDR bacteria. No pathological abnormalities were noticed even if it was provided at the highest treatment dose of 800 mg/kg body weight. Further In silico study of molecular interaction analyses have been performed for the proteins Extracellular Adhesion Protein (EAP), Protein A, and Toxic Shock Syndrome Toxin (TSST) of *Staphylococcus aureus* with the ligand 2-methoxyethyl benzene which shows better interaction with the target. This study validated the robust antibacterial activity of the essential oil of *P. odorifer* against both gram-positive and negative bacteria. Further, it is validated that the antibacterial activity was mainly because of the presence of two major phytochemicals, PEME and terpineol identified by HRMS analysis.

Keywords: Antibacterial activity; HRMS analysis; Medicinal plants; Secondary metabolites

INTRODUCTION

Medicinal & Aromatic Plants (MAPs) are organic raw materials, usually referred to as natural pharmaceuticals, that are primarily used as constituents in cosmetics, health, medicinal products, and other natural health products for therapeutic, aromatic, and culinary purposes. (**Zhou *et al.*, 2022; Boukhatem and Setzer, 2020; Fierascu *et al.*, 2021**). Plant extracts and essential oils (EOs) are sources of advantageous chemical compounds that have the potential to be used in agriculture, food, cosmetics, and medicine. For some time, the only method for preventing and treating human ailments was plant-based medicine. Ingredients derived from plants thus become essential for preparing natural medications. The first crucial step in making these compounds is the extraction of the EOs. These chemicals can be developed effectively using conventional extraction techniques. Additionally, the substances derived from plants naturally fight off a variety of pathogens that tend to cause disease. (**Boukhatem and Setzer, 2020; Fierascu *et al.*, 2021**). Aromatherapy and the treatment of ailments such as cardiovascular disease, diabetes, Alzheimer's, and cancer use essential oils. (**Sharifi-Rad *et al.*, 2017**). Additionally, several active substances are found in EOs, including alkaloid substances, tannins, steroids, glycosides, resins, phenols, volatile oils, and flavonoids. In response to consumer concerns about the negative effects of synthetic antioxidants, which have hazardous side effects on humans and subsequently cause numerous illnesses, EOs are currently gaining prominence as natural alternatives to synthetic antioxidants (**Amirifar *et al.*, 2022; Asgari *et al.*, 2017**) Synergistic implications encompass scenes when it is combining two or more substances results in increased activity compared to when these substances are used separately. (**Ju *et al.*, 2022**). A natural plant-derived essential oil has a huge potential for use as an alternative to chemical-based antibiotic compounds because they impair health and cause problems including the emergence of antibiotic-resistant bacterial strains. (**Li *et al.*, 2022**). Furthermore, using synthetic compounds to control infectious microorganisms is perilous due to its potential for hazards to the environment, acute toxicity, and carcinogenic effects. In this way, managing multidrug-resistant pathogenic bacteria with essential oils can aid in the treatment of several infectious diseases (**El-Zehery *et al.*,2022**).

The aromatic plant *Pandanus odorifer* commonly known as kewda belongs to the monocot species of the family Pandanaceaeand is found all over the world (**Adkar and Bhaskar, 2014; Singh and Parle, 2015; Nasim *et al.*, 2018**). Pandanus fruit pulp comprises provitamin-A carotenoid. It can benefit individuals who are deficient in vitamin A. Carotenoids contained in food prevent cancer, diabetes, and heart disease. A rich supply of vitamin C (ascorbic acid), thiamine, riboflavin, and niacin (vitamin B3) are also found in pandanus fruit. (**Adkar and Bhaskar, 2014; Singh and Parle, 2015; Nasim *et al.*,2018**). Through extensive research work has been carried out on this plant species, just a fraction is known about the essential oil of Pandanus sp.'s antimicrobial attributes. Hence, in this study, attempts have been made to evaluate the antibacterial activity of essential oil extracted from the flowers of *P. odorifer*. Further, the presence of different phytochemicals in the essential oil was identified based on HRMS analysis.

MATERIAL AND METHODS

Essential oil xtraction

After removing the green sections, the *P. odorifer* blooms were collected and cut into smaller pieces that were between one and two inches long. The 5-litre round bottom flask was filled with water and the chopped flowers in a 1:3 ratio, and Clevenger's apparatus was then attached. For three hours at 60–70 0C, the flask was left on the heating mantle for hydro-distillation. In the first distillate, which is produced after an hour at a temperature of around 100 to 110 0C and about 80% of the essential oil is extracted. After a 3-hour distillation period, the remaining oil is extracted. The extracted Kewda essential oil with scent was placed in airtight glass vials for storage once hydrodistillation was finished. Phenyl Ethyl Methyl Ether and Terpineol, two important phytochemicals of essential oils, were purchased from Sigma Aldrich to test their antibacterial activities.

Detection of antibacterial activity

The antibacterial properties of the derived essential oil (20 g/ml) were evaluated using the agar well diffusion method, and two of its main phytochemicals, PEME (5 g/ml) and Terpinol (5 g/ml). The Micro dilution approach was used to calculate the minimal inhibitory concentration (Aziz et al., 2018). The Department of Microbiology, IMS, and SUM Hospital, Bhubaneswar, Odisha, India, provided the four identified MDR bacterial *strains Staphylococcus aureus*, *Streptococcus pyogenes*, *Acinetobacter baumanni*, and *Pseudomonas aeruginosa*, which were used to test the antibacterial activity (Aziz et al., 2018). The control strains employed were the standard MTCC strains for S. aureus (MTCC 7443), S. pyogenes (MTCC 1928), *A. baumanni* (MTCC 1425), and *P. aeruginosa* (MTCC 1688).

The antibacterial property was evaluated using Agarwell diffusion method. A 100 l nutrient agar plate with a fully developed broth culture of four bacterial strains was disseminated. Four 0.5 mm-diameter wells were drilled into each culture plate using a sterile cork borer, and 50 l each of essential oil (20 g/ml), PEME (5 g/ml), terpinol (5 g/ml), and linezolid/imipenem (30 g/ml or 10 g/ml) were added. For 30 minutes, the compounds were allowed to diffuse in the laminar airflow at ambient temperature. Inoculums for the control studies only included 10% DMSO. The plates were then incubated for 18 to 24 hours at a temperature of 37 0C. After the procedure, Following the period of incubation, the diameter of the inhibitory zone (in mm) was determined. The studies had all been carried out in triplicate (n = 3).

Detection of minimum inhibitory concentration

The micro broth dilution method, which was previously described (Dubey et al., 2022; Pradhan et al.,2022), was used to evaluate the minimum inhibitory concentration (MIC) of the extracted essential oil, PEME, and Terpinol. Briefly, 50 l of broth media, gradient concentrations of extracted essential oils or two of its main phytochemicals, PEME or Terpineol, 10 l of resazurin indicator solution, and 10 l of bacterial suspension ($5x10^6$ CFU/ml) were placed into sterile 96-well plates. Two blanks were taken, from which neither bacterial culture nor any of the well's primary phytochemicals were added to any of the two wells. The duplicate plates were produced and incubated at 37 °C for 18 to 24 hours. the transition from purple to the presence of germs was noted as being pink or colourless. The minimum inhibitory concentration (MIC) value is the lowest concentration at which the colour did not change and stayed blue. As a standard, piperacillin and the broad-spectrum antibiotic Tazobactam were taken. For each of the four bacterial strains, the MIC value for the control was individually calculated. Given that it is a widely used standard antibiotic, 1.0 mg/ml stock solution and gradient concentrations were made as necessary.

Detection of the antimicrobial compound

The Exactive Plus Orbitrap high-resolution mass spectrometer and Ultimate 3000 high-performance liquid chromatography (both from Thermo Scientific, USA) were used to undertake a perceptible investigation of the phytochemical constituent of the extracted essential oil. Fisher Scientific (USA) provided the LC-MS grade methanol, water, formic acid, acetonitrile, and acetone. 1.5 ml of a 1:1 methanol: water solution and 0.5 ml of the extracted essential oil were added to a 20 ml centrifuge tube. To get rid of any solid particles, the solution was allowed to be centrifuged for 10 minutes while being swiftly mixed at 4^0C and 10,000 rpm. After that, A 0.22-M syringe filter was used to filter the solution to get eliminate any additional debris. The filtrate was aspirated and 0.5mL was placed in the DP ID vial. For mass spectrometry, use (Cat#C4000-1W, Thermo Scientific, USA). To separate the phytochemicals in the solution, a Hypersil BDS C18 (250 mm 2.1 mm, 5 m; Thermo Scientific, USA) column was employed. The mobile phage is made out of a 1:1 methanol: water solution with 0.1% formic acid. The sample flow rate was set to 3 l/min, the column temperature was kept at 30, and the pressure remained at 700 bar. The compound was introduced into the orbitrap chamber through nitrogen gas and ionised using an electrospray ionisation method at a voltage of 3eV. With a scan range of 50-750 m/z, the ions were found based on the polarity of positive as well as negative after the sample was run for 15 minutes. The standard curve was used to compare the mass peak intensities. It was created by transforming peak intensities into concentration by averaging the peak magnitudes of the five benchmark molecules with masses between 50 and 500 m/z. After parsing the mass peaks using a custom Python programme with an error range of 0.01 m/z, the mass peaks were subsequently annotated (**Behera *et al.*, 2022**).

Toxicity study

The male and female rats (120-150g), aged 6-8 weeks were purchased from Neelachal tirati, Kolkata, Saha enterprises(1828/po/Bt/S/15/CPCSEA). The institutional animal ethics committee gave its approval to the methods of experimentation. of the School of Pharmaceutical Sciences, Sikha 'O' Aunsandhan University, Bhubaneswar, Odisha (ProtocolIAEC/SPS/SOA/18/2019). Experiments were conducted in the lab having registration no 1171/To/Re/S/08/TPCSPAThe animals were housed in an iron cage system in a comfortable habitat with a 12-hour light/dark cycle. Feeding the animals were provided with water regularly. Before beginning the investigation, the animals were acclimated for a week.

Weighing adequate amounts of kewda oil (200, 400, and 800 mg/kg) was used to make a solution. It was then dissolved in the appropriate amounts of water to achieve the concentrations sought for each treatment group. Because oil is insoluble in water, it was first dissolved in 0.1% tween 20, a surfactant, and then in water.

Forty rats were randomly divided into four groups, each group containing ten animals five male and five female**.** The control group received water only, whereas the treated group received the essential oils by cannula. After administering the essential oils, the animals were observed for any changes in the spontaneous type, alertness, awareness, sound response, touch response, pain response, righting reflex, pinna reflex, grip strength, food intake, water intake general behaviour, physiological activities, and survival.

After 45 days, the animals were anaesthetized with formalin and were sacrificed to collect their blood and organs (liver, kidney) for biochemical and histological analysis. A heart puncture was used to draw blood samples, which were then collected in a sterile tube for biochemical analysis. The assay kit was used to measure glucose (G), total cholesterol (TC), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea (Ur), creatinine (Cr), and total protein from serum. The organs were preserved in 10% formaldehyde and processed for histological study by making paraffin blocks. Histopathological analysis was carried out by measuring physical and biochemical parameters like tissue injury, necrosis, apoptosis and inflammation by staining with haematoxylin and eosin. The OECD criteria of 423 and 407 were followed for conducting both acute and subacute toxicity analyses. Animals received doses of 200, 400, and 800 mg/kg body weight.

Molecular docking study

The targeted proteins of the MDR strain, *Staphylococcus aureus* subsp. *aureus* Mu50 and *Staphylococcus aureus* subsp. *aureus* COL protein 3D structure was retrieved from Protein Data Bank (PDB) https://www.rcsb.org/. The structural information of the PEME *(*2-methoxyethyl benzene*)* 3D structure was downloaded from PubChem (http://www.ncbi.nlm.nih.gov/pccompound). The AutoDock 4.2.6 software was used for the molecular docking process and the Pymol software was used for the study of protein-ligand interactions and visualization. In this study, the protein of the selected MDR strains (which showed maximum antibacterial activity) and ligand PEME were docked. The obtained docking score values could be used to predict the individual efficacy of each protein. The docking score was calculated for MDR strain (**Meher *et al.*, 2021a; Meher *et al.*, 2021b**).

Statistics

The data were statistically analysed using the mean and standard deviation. The data collected from three different studies were used to compute the mean values and standard deviations. A significant statistical difference was defined as $p < 0.05$. (**Meher *et al.*, 2021c**).

RESULTS AND DISCUSSION

Evaluation of antibacterial activity

*P. odorifer*a high-value There are several therapeutic advantages of medicinal plants, including analgesic, cancer-preventing, antidiabetic, antimicrobial, anticonvulsant, antioxidant, antidepressant, and anti-neurotic action. It is mostly made up of secondary metabolites such as steroids, terpenoids, lipids, coumarins, alkaloids, flavonoids, phenols, and lignans (**Hussain *et al.*, 2019**). We investigated the antibacterial activity of the essential oil and two of its major phytochemicals, PEME and Terpineol by the existence or absence of an inhibitory zone **(Table 1)** against both Gram-positive and Gram-negative MDR bacteria mainly because of the exponential rise in MDR bacteria strain against the currently used antibiotics (**Qin *et al.*, 2022; Chiabchalard and Nooron, 2015**). The essential oil revealed significant antibacterial activity against 4 bacteria and formed a clear zone of inhibition. It showed a zone of inhibition of 32 mm against *S. aureus*, 29 mm against *S. pyogenes*, 29 mm against *A. baumanni*, and 28 mm against *P. aeruginosa*. Similarly, PEME showed a 29 mm zone of inhibition against *S. aureus*,26 mm against both *S. pyogenes* and *A. baumanni* and 25mm against *P. aeruginosa*.

Table 1 Antimicrobial assay by agar well diffusion method of *P.odorifer* essential oil, PEME, and Terpinol against MDR bacterial strains (zone of inhibition in mm)**.**

Strain	Essential oil 20 µg/ml	PEME 5 µg/ml	Terpinol 5 µg/ml	Linezolid/ imipenem (30/10µg/ml)
Staphylococcus aureus	32	29	27	29
Streptococcus pyogenes	29	26	22	29
Acinetobacter baumanni	29	26	24	33
Pseudomonas aeruginosa	28	25	27	26

Parenthetically minimum MIC and MBC value of the essential oil was found against 4 bacterial strains **(Table 2).** The lowest MIC and MBC values of 0.078 and 1.25 µg/ml were noted against *S. aureus* and the highest value of 0.62 and 2.5 µg/ml against *S. pyogenes*, *A. baumanni*, and *P. aeruginosa*. In contrast, PEME revealed the lowest MIC and MBC values of 0.31 and 2.5 µg/ml against *S. aureus* and the highest value of 0.62 and 2.5 µg/ml against *S. pyogenes*, *A. baumanni* and *P. aeruginosa* (**Table 2**). Similarly, Terpineol showed MIC and MBC values of 0.31 and 2.5 µg/ml against *S. aureus*, whereas the highest value of 0.62 and 0.25 µg/ml were against *S. pyogenes* (**Table 2**). The significant antibacterial activity noted with the essential oil and two of its major phytochemicals can assume a pivotal part in controlling MDR bacterial strains where the majority of the synthetic-based antimicrobial drugs may have serious side effects.

Table 2 MIC and MBC of *P. odorifer* essential oil, PEME, and Terpineol against MDR bacterial strains

Strain	Crude oil (20 µg/ml)		PEME (10µg/ml)		Terpinol (10 µg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	0.078	1.25	0.31	1.25	0.31	2.5
Streptococcus pyogenes	0.62	2.5	0.62	1.25	0.62	2.5
Acinetobacter baumanni	0.62	2.5	0.62	2.5	0.15	1.25
Pseudomonas aeruginosa	0.62	2.5	0.62	2.5	0.31	1.25

Legends: MIC*: minimum inhibitory concentration*; MBC: *minimum bactericidal concentration.*

HRMS Analysis of crude essential oil of P. odorifer

The phytochemical screening of the essential oil of *P. odorifer* revealed the presence of various secondary metabolites (**Table 3**). Different phytochemicals in the essential oil were identified on the analysis of the fragmentation pattern of mass spectra and direct comparison of their spectral data with the chemical profiles, using the NIST library, and by comparisons of published mass spectra (**Figure 1**).

Table 3 Phytochemical constituents identified in the essential oil of *P. odorifer* by HRMS analysis.

Sl.No	Compound name	Molecular weight	Concentration (pico-mole)
1.	p-benzoquinone	108.0211294	31.641
2.	2-phenethyl alcohol	122.0731649	14.569
3.	p-cymene	134.1095505	48.500
4.	2-phenethyl methyl ether	136.088815	112.22
5.	alpha-Terpineol	154.1357652	221.24
6.	Psoralen	186.0316941	20.377
7.	Isoplumbagin	188.0473441	2.3489
8.	Genipin	226.0841236	1.2797
9.	Artemidiol	234.0892089	5.6275
10.	Pinocembrin	256.0735589	0.5880
11.	(-)-Glycinol	272.0684735	1.75057
12.	Iprobenfos	288.0949027	0.4641

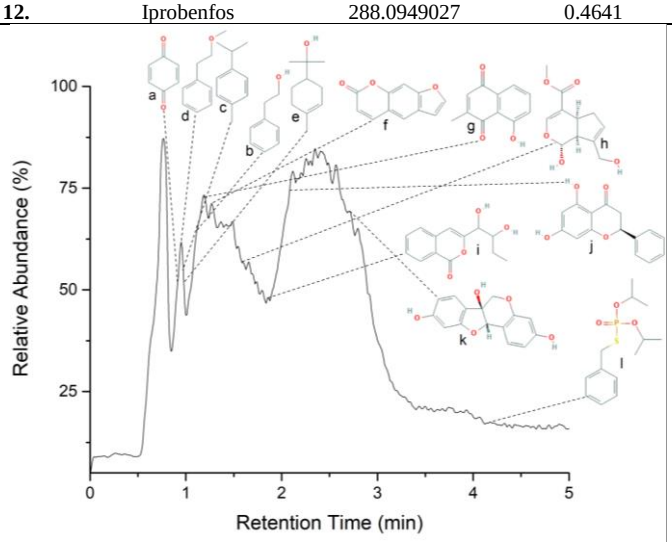

Figure 1 Mass spectra of the essential oil of *P. odorifer* indicate the presence of several major phytochemicals.

The HR-MS analysis of essential oil identified the presence of 12 major phytochemicals namely p-benzoquinone, 2-phenethyl alcohol, p-cymene, 2-phenethyl methyl ether alpha-Terpineol, Psoralen, Isoplumbagin, Genipin, Artemidiol, Pinocembrin, (-)-Glycinol, and Iprobenfos. The components were reported to be with sufficient antibacterial activity as reported by authors who had taken an interest to study the antibacterial activity of individual constituents (**Pattnaik *et al.*, 1997**).

Toxicity study

The treated animals with the essential oil of *P. odorifer* showed neither any toxic effect nor any lethal effect. Even the administration of the highest dose up to 800mg/kg did not reveal any signs of toxicity or mortality in rats during the entire period of study. Therefore, the LD_{50} of the essential oil was considered to be greater than 800mg/kg. No significant difference in organ functions between the treated and untreated groups (**Table 4**) was noticed. Also during the dosage regimen, no abnormal behaviour regarding food and water consumption and body weight was observed. A non-significant decrease($p>0.05$) in ALT and AST was observed which are markers for liver injury. This indicated that kewda oil has no toxic effect on the liver.

Table 4 Observations on the normal symptoms of the animals with the treatment of different doses of essential oil of *P. odorifer*.

Behaviour Type	Control and treated groups of animals			
	Control	200 mg/kg	400 mg/kg	800 mg/kg
Spontaneous type	Nor	Nor	Nor	Nor
Alertness	Nor	Nor	Nor	Nor
Awareness	Nor	Nor	Nor	Nor
Sound response	Nor	Nor	Nor	Nor
Touch response	Nor	Nor	Nor	Nor
Pain response	Nor	Nor	Nor	Nor
Righting reflex	Nor	Nor	Nor	Nor
Pinna reflex	Nor	Nor	Nor	Nor
Grip strength	Nor	Nor	Nor	Nor
Food intake	Nor	Nor	Nor	Nor
Water intake	Nor	Nor	Nor	Nor
Mortality	Abs	Abs	Abs	Abs

Urea levels remained unchanged while creatinine levels in blood were slightly reduced after the administration of oil in all treatment groups (**Table 5**)**.** Statistical analysis revealed a nonsignificant alternation ($p>0.05$) in triglycerides and cholesterol values in groups. Glucose and protein levels increased slightly.

According to **Nisha *et al.* (2017),** renal toxicity should be considered only when creatinine and urea levels increased parallel to each other**.** To identify hyperlipidemia total cholesterol and triglycerides were evaluated. Which are risk factors for heart disease. Both cholesterol and triglycerides were lying in between the normal range. Nor: Normal; Abs: Absent

Table-5- Blood biochemical parameters and organ functions between the control and the treated groups with different doses of essential oil of *P. odorifer***.**

Parameters	Group-I	Group-II	Group-III	Group-IV	Normal range
Glucose(mg/dl)	82.05±2.1	82.89±1.98	83.02±1.4	82.93±1.9	70-110
Urea(mg/dl)	30.02±3.1	30.04±2.98	30.86±1.78	30.29±2.14	15-45
Creatinine(mg/dl)	0.78±1.05	0.72±1.34	0.72±1.25	0.73±1.47	0.5-1.5
Total protein(mg/dl)	5.98±2.56	6.18±1.45	6.26±1.76	6.14±1.5	6.0-8.0
Total cholesterol(mg/dl)	126.02±1.78	126.32±2.22	126.89±2.3	126.87±2.5	140-250
Tri glycerides(mg/dl)	89.02±3.1	89.01±2.9	90.01±2.8	90.56±2.6	25-160
Asparateaminotransferase(AST)(IU/L)	30.02±2.5	29.99±2.6	29.32±1.9	29.12±3.2	Up to 46
Alanine aminotransferase (ALT)(IU/L)	25.89±2.1	25.27±1.6	25.12±1.9	25.39±2.2	Up to 40

Legends: Group I:-Control, Group II:-200mg/kg body wt, Group III:-400mg/kg body wt, Group IV:-800mg/kg body wt

We tested the liver and kidneys of rats to see if essential oil treatment causes toxicity to normal tissues. H&E staining of paraffin-embedded 5-micron-thick liver and kidney sections at a magnification of 200x (Figure 2). Essential oil treatment at doses ranging from 200 mg/kg to 800 mg/kg fails to reveal any detectable pathological changes in the liver or kidney. The hepatic lobular architecture was normal. Normal glomeruli, proximal and distal tubules, interstitium, and blood arteries were found in the kidneys. We discovered that the LD_{50} value for Kewda oil was greater than 800mg/kg body weight in this study, but **Tabarraei et al. (2019)** investigated the acute toxicity of black caraway seed essential oil in Wistar rats by administering 500, 1000, 1500, 2000, 2500, 3000, 3500, and 4000 mg/kg seed essential oil and discovered that the LD_{50} value for black caraway seed essential oil was greater than 4000 mg/kg body weight. At the same time, sub-acute toxicity studies reveal that black caraway essential oil has no effect on the body's important organs. **Mekonnen et al. (2019),** on the other hand, reported an LD50 value for L. angustifolia essential oil that was greater than 2000 mg/kg. The histopathology study of kidneys and livers revealed no macroscopic alterations at this level of dosage. While **Andrade et al. (2014)** revealed Doses of 30, 60, and 120mg/kg of the essential oil of *Lippia origanoides* did not show any histopathological changes to the liver, kidneys, or heart of the treated rats.

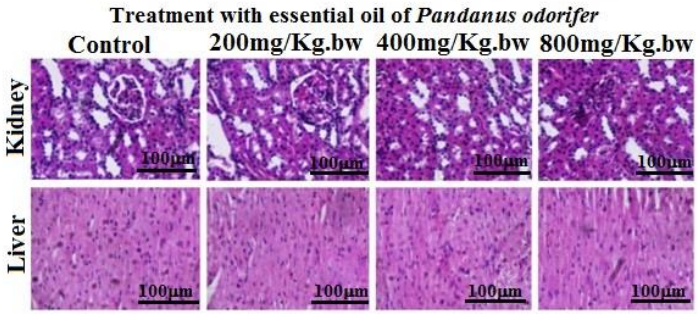

Figure 2 Panels represent H&E staining of paraffin-embedded 5-micron-thick sections of the liver and, kidney at a magnification of 200x and scale bar of 100µm.

Molecular interaction between protein and ligand

Molecular interaction analyses have been performed for the proteins Extracellular Adhesion Protein (EAP), Protein A, and Toxic Shock Syndrome Toxin (TSST) of *Staphylococcus aureus* with the ligand 2-methoxyethyl benzene. In all the complex structures of EAP, protein A, and TSST proteins, the ligand 2-methoxyethyl benzene is bound within a hydrophobic cleft (**Figure 3a-c**). It has also been noticed that all the nonbonded interactions between the proteins and ligands have taken place with the help of the only oxygen atom situated in the methoxyethyl tail of the ligand. In the case of the EAP-ligand complex, two non-bonded interactions are formed between the oxygen atom of the ligand and two side-chain atoms, OD1 and OD2 of Asp122 of the EAP enzyme with distances of 3.4 and 3.9 Å respectively (**Figure 3a**). While in the Protein A-ligand complex, only one molecular interaction has been reported between the side-chain oxygen atom of Ser2831 of the protein and the ligand oxygen atom with a bond distance of 2.8 Å (**Figure 3b**). Interestingly, the highest number of polar interactions (4 numbers) have been recorded in the complex structure of TSST protein with the ligand. In this complex structure, all the molecular interactions have been formed between the main chain atom of the residues *viz.* Tyr51, Tyr52, and Ser53 of the enzyme. The oxygen atom of the ligand interacts with the main chain oxygen and the nitrogen atom of Tyr51 and Tyr52 with a bond distance of 4.0 and 3.3 Å respectively. There are two polar interactions have been reported between the oxygen atom of the ligand with both the main chain oxygen and nitrogen atoms of Ser53 with a bond distance of 3.5 and 2.9 Å respectively (**Figure 3c**).

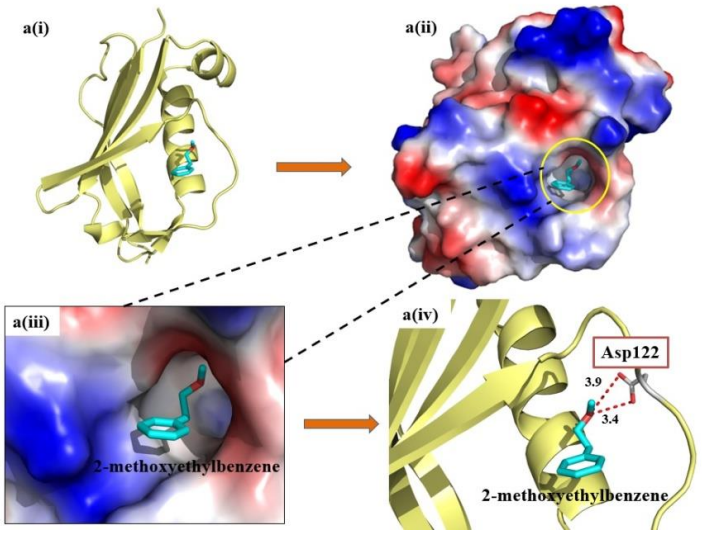

Figure 3a Complexed structure of Extracellular Adhesion Protein (EAP) of *Staphylococcus aureus* with 2-methoxyethyl benzene [an (i)], Electrostatic surface potential of that complexed structure [an (ii)], Electrostatic surface potential of 2-methoxyethyl benzene binding cleft in *S. aureus* EAP [an (iii)], Molecular interaction between *S. aureus* EAP and 2-methoxyethyl benzene [an (iv)].

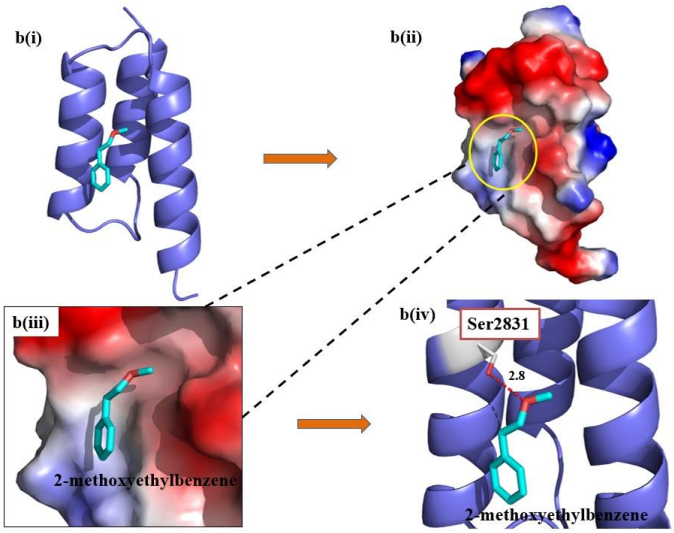

Figure 3b Complexed structure of Protein A of *Staphylococcus aureus* with 2-methoxyethyl benzene [b(i)], Electrostatic surface potential of that complexed structure [b(ii)], Electrostatic surface potential of 2-methoxyethyl benzene binding cleft in *S. aureus* Protein A [b(iii)], Molecular interaction between *S. aureus* Protein A and 2-methoxyethyl benzene [b(iv)].

From the comparative molecular interaction analysis between the 3 proteins *viz.* EAP, ProteinA, and TSST of *S. aureus* with the ligand 2-methoxyethyl benzene, it has been revealed that hydrophobic interactions play a major role in ligand binding

with all the receptor enzymes studied here. Among these 3 enzymes, TSST was shown to possess the maximum number of non-bonded interactions with the ligand 2-methoxyethyl benzene and depicted the highest binding affinity with the enzyme. It is also noteworthy that in the TSST-ligand complex, all the non-bonded interactions have been formed with the help of the main chain atoms of protein residues which might assist the formation of a strong complex between protein and ligand as very less positional fluctuations have occurred in those main chains interacting atoms in the enzyme. **Paul *et al.* (2020),** in their study, has also shown that hydrophobic interactions play an important role in the interactions between cellulosic substrate like β-D glucose in the binding cleft of microbial cellulases.

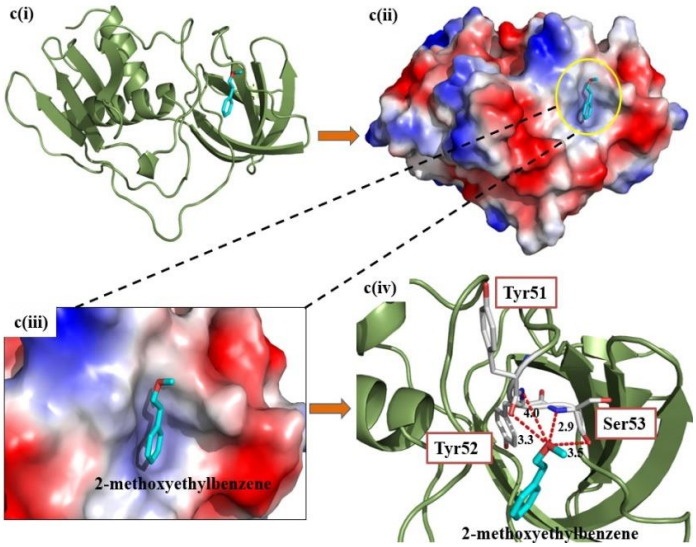

Figure 3c Complexed structure of Toxic Shock Syndrome Toxin (TSST) of *Staphylococcus aureus* with 2-methoxyethyl benzene [c(i)], Electrostatic surface potential of that complexed structure [c(ii)], Electrostatic surface potential of 2-methoxyethyl benzene binding cleft in *S. aureus* TSST [c(iii)], Molecular interaction between *S. aureus* TSST and 2-methoxyethyl benzene [c(iv)].

CONCLUSION

The current investigation validated the antibacterial activity of essential oil derived from *P.odorifer* against both gram-positive and negative bacteria and the HRMS analysis revealed the presence of various secondary metabolites which can be attributed to the antibacterial activity of this plant. Further toxicological investigation of *P. odorifer* did not show any adverse effect on the biochemical and histological parameters related to living and kidney function tests up to the high concentration dose of 800mg/kg body weight for 45 days. Thus based on the current *in vivo*, *in vitro* and *in silico* study report, it was concluded that kewda oil is a potential antibacterial agent against the pathogenic bacterial strain without causing any toxicity to animals on the above-tested dose.

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