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RESEARCH ARTICLE

Characterization of bioactive compounds from *Saraca asoca* and their antibacterial activity against fish pathogens in *Oreochromis niloticus*

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Abstract

Saraca asoca, known for its therapeutic properties in Ayurveda, is the focus of this study, aiming to identify and quantify the bioactive compounds in its leaf extract using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR). The study also investigates the antibacterial efficacy of methanol, ethanol and acetone extracts of *S. asoca* against fish pathogens in *Oreochromis niloticus* like *Vibrio alginolyticus*, *Streptococcus pyogenes*, *Pseudomonas fluorescens* and *Aeromonas hydrophila* through the well-diffusion method. GC-MS confirmed the presence of compounds such as 3- hydroxy biphenyl, n-hexa decanoic acid, oleic acid, octadecanoic acid, 4,5-diethyl octane and 9-tetradecen-1-ol. In contrast, FTIR spectra revealed several significant peaks, indicating the presence of specific functional groups in the *S. asoca* leaf fraction. The results exhibited high absorbance in the wavenumber ranges of 4000-3500 cm^{-1}, 3000-2500 cm^{-1}, 1800-1500 cm^{-1} and 1100-950 cm^{-1}. The findings of the antibacterial assay suggest that the methanolic extract exhibited a strong inhibitory effect against bacterial pathogens, with zones of inhibition ranging from 6 ± 0.21 to 18 ± 0.57 mm in size. These results indicate that *S. asoca* leaf extract contains bioactive compounds effective against the pathogenic bacteria in *O. niloticus*, supporting the growing shift towards reducing antibiotic use in aquaculture.

Keywords: antibacterial activity; bacterial pathogens; bioactive compounds; *Oreochromis niloticus*; *Saraca asoca*

Introduction

Fish consumption worldwide grew by 3.1 % annually between 1961 and 2017. During the same period, this was almost twice as fast as the global populations' 1.6 % annual growth rate and more than the 2.1 % growth rate for all other foods containing animal protein (meat, dairy, milk, etc.) (1). According to the FAO, global fisheries and aquaculture production increased from 110.7 M mt in 1990 to 178.9 M mt in 2018, slightly declining to 1.5 M mt in 2019 and 1.1 M mt in 2020 (2). Asia has produced 89 % of the worlds' fish in volume over the last 20 years, making it the global leader in this field (3). India has a promising contribution to global aquaculture production. Indias' fish output increased steadily from the 1980s to 2018, growing from 1.69 million tonnes to 4 million tonnes, demonstrating substantial industry expansion (4). Maintaining this growth will be vital for addressing domestic needs and tapping into export markets (5).

One of the significant challenges in the aquaculture industry is the prevention of infectious diseases, which is considered a global public threat (6). Infectious diseases are driven by the rise of new pathogens through genetic mutations, zoonotic transmission, environmental shifts, international travel, antibiotic resistance, genetic reassortment and changes in human behavior (7). Infections, primarily bacterial infections, cause significant losses in tilapia farming, one of the second most important farmed fish globally (8). For decades, some bacterial species belonging to at least four genera have been considered essential pathogens for Nile tilapia, *Oreochromis niloticus*: *Streptococcus* species, motile *Aeromonas* spp., *Flavobacterium* spp., *Edwardsiella* spp. and *Vibrio* species (9). *Vibrio alginolyticus*, a gram-negative, rod-shaped, motile, single-polar flagellum bacterium commonly found in marine environments, particularly in coastal waters, causes vibriosis in farmed tilapia (10). *Streptococcus pyrogens*, or Group A *Streptococcus* (GAS), are gram-positive spherical (cocci) and often occur in chains. Reports are showing streptococcal infections in tilapia farms (11). *Pseudomonas fluorescens* causes septicemia illnesses, another prevalent infection affecting *O. niloticus*. These can cause clinical signs such as skin hemorrhage, fin rot, detached scales, ascites and exophthalmia in fish species (12). *Aeromonas hydrophila*, a gram-negative bacteria abundant in aquatic settings with wide distribution, is also an opportunistic pathogen causing significant mortalities among farm and wild fishes (13).

Previous studies reported that introducing antibiotics to aquaculture could inhibit infectious diseases in fish (14). However, the evolving novel mutant strains and the increasing incidence of drug-resistant infections have considerably reduced the efficiency of antibiotics in controlling aquatic diseases (15). These findings highlight the limits of traditional techniques, which frequently fail to prevent the spread of illnesses in marine environments. Several vaccines have been created in response to these issues, utilizing modern molecular technology to give more focused and effective options for preventing and treating aquatic diseases (16). Vaccines in aquaculture are primarily based on Pasteurian principles, which involve the isolation, inactivation, or destruction of pathogens before developing a vaccine (17). This vaccine can then be administered through various delivery techniques, such as immersion, injection and oral vaccination (18). These innovative vaccines utilize cutting-edge techniques such as molecular cloning, DNA and RNA vaccine platforms and viral vector technologies, offering enhanced specificity and efficacy in combating known and emerging pathogens (19). The major disadvantage of emerging vaccination technologies in aquaculture and veterinary medicine lies in scalability, cost-effectiveness and the need for extensive species-specific validation. Therefore, plants are promising and innovative in aquaculture, with significant potential for further research and development (20).

Evaluating plants for use in aquaculture is a comprehensive procedure that needs a detailed study of their chemical composition, phytochemical characteristics, antibacterial capabilities, toxicity, bioavailability and effectiveness under field circumstances (21). Phytochemicals, such as alkaloids, flavonoids, tannins and terpenoids in these plants underscore their potential in treating infectious diseases in aquaculture (22). Identifying bioactive compounds and *in vitro* and *in vivo* testing helps determine the plants' potential as a natural alternative to synthetic antibiotics (23). Antibacterial tests are critical for finding and confirming the medicinal potential of plant extracts, particularly with the rising issue of antibiotic resistance (24). These tests confirm traditional plant usage, making it easier to produce standardized medicinal formulations and directing the selection of the most effective compounds.

S. asoca is a medicinal plant known for its pharmacological importance. It has been proven to have a role in cardio-protective, anti-inflammatory, antioxidant, anticarcinogenic and radical scavenging activities (25). This study aims to identify and quantify the chemical compounds in *S. asoca* using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR) while evaluating the antibacterial effects of methanol, ethanol and acetone extracts against four fish pathogenic bacteria strains (*V. alginolyticus*, *S. pyogenes*, *P. fluorescens* and *A. hydrophila*), which are associated with diseases in Nile tilapia, *O. niloticus*. This study aims to investigate the bioactive constituents in *S. asoca* and its potential as a natural alternative to synthetic antibiotics in aquaculture, therefore contributing to the development of long-term and effective treatment options for fish health.

Materials and Methods

Collection of plant material and preparation of extract

S. asoca leaves were collected from Thiruvananthapuram, Kerala (Fig. 1) and identified to avoid misinterpreting the plants of interest with similar-looking plants. The plant authentication number was given in our article (26). The plant extract was prepared using three different solvents: methanol, ethanol and acetone. About 5 g of the dried powder of the *S. asoca* leaves was taken in test tubes separately with 20 mL of three different solvents. After 48 hr, the solution was filtered through Whatman No. 1 paper to allow complete solvent drainage. The extract was then resuspended in the extracting solvent to form a stock solution of 500 mg/mL and further diluted according to the experimental design (27).

Characterization of *S. asoca* extracts

GC-MS analysis

The GC-MS analysis of *S. asoca* whole plant extract was conducted using the Shimadzu GC-MS-QP2010 SE. The auto-sampler executed two rinses with pre-solvent, post-solvent and the sample itself, employing high plunger speeds for suction, injection and washing, with a viscosity compensation time of 0.2 sec. The injection mode was normal, featuring five pumping cycles and an injection port dwell time of 0.3 sec, using one solvent vial and no terminal air gap. The GC-2010 operated in split-less injection mode at 280 °C, with a sampling duration of 1.00 min, a linear velocity flow control mode at 76.2 kPa pressure, a total flow of 4.3 mL/min and a column flow of 1.25 mL/min. The oven temperature program started at 60 °C, held for 3 min, then increased to 200 °C at 12° C/min (held for 2 min) and finally rose to 280 °C at 25 °C/min (held for 16 min), with a 3 min equilibrium time. The GCMS-QP2010 SE was set with an ion source temperature of 200 °C, an interface temperature of 290 °C and a solvent cut time of 3 min. The mass spectrometer operated in scan mode, ranging from 35 to 500 m/z, with a scan speed of 1666, an event time of 0.30 sec and the detector gain set at +0.00 kV relative to the tuning result. The constituents were identified upon comparing the existing library (Library: NIST17.lib) and results have been tabulated.

Fig. 1. Morphology of *S. asoca* leaves.

FT-IR analysis

The Fourier Transform Infrared Spectrophotometer (FTIR) is one of the most effective tools for identifying the types of chemical bonds, or functional groups, in compounds. The wavelength of light absorbed is specific to each chemical bond, as illustrated in the annotated spectrum. A powdered sample of the plant was analyzed using an FTIR spectrophotometer (Shimadzu, IR Affinity 1, Japan) with a scan range of 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1}. The prominent bands observed in the FTIR results were compared with standard reference values to identify the functional groups of the chemical compounds present in the leaf of *S. asoca*.

Antibacterial activity of *S. asoca* extracts

Test microorganisms and their inoculum preparation

The pure culture of the test organisms was sourced by the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. *V. alginolyticus* (MTCC 13127), *S. pyrogenes* (MTCC 442), *P. fluorescens* (MTCC 103) and *A. hydrophila* (MTCC 12301) were the bacterial strains employed in the investigation. 38 g of Mueller-Hinton Broth (MHB) media was dissolved in 1 L of distilled water (28). With frequent agitation, the media was boiled for 1 min to dissolve completely and it was autoclaved at 121 °C for 15 min and later cooled to room temperature (The final pH was maintained at 7.3 ± 0.1 at 25 °C). The bacterial strains were individually inoculated into MHB and kept as a starter culture. After 24 hr, they were plated in Muller Hinton agar (MHA) for the antibacterial assay.

Stock preparation of plant extract

To prepare the stock solution of plant extract and various extract concentrations, 500 mg of each extract was dissolved in 5 mL of the corresponding extract in a test tube, resulting in a 100 mg/mL concentration. Serial dilutions were then performed by transferring 0.5 mL of this stock solution to a new test tube containing 1 mL of distilled water, producing a 50 mg/mL concentration. This process was repeated to achieve further diluted concentrations.

Antibacterial activity (Well diffusion method)

Well diffusion assays were carried out according to a standard protocol with some modifications (29). Bacteria colonies from plates were grown in MHB until they reached their specific OD at 600 nm to give a starting inoculum of 1×10^8 bacteria/mL. 200 µL of the bacterial samples were inoculated into the petri plates using a sterile, autoclaved L-shaped glass rod and spread uniformly. Six wells were punched on each plate and labeled accordingly. 50 µL of methanol, ethanol and acetone extract from the plant sample was loaded into each of the wells. The solvents used for extraction, like methanol, ethanol and acetone, were used as the negative control and a standard antibiotic as the positive control.

Determination of minimum inhibitory concentration (MIC)

The MIC is the lowest concentration of plant extract that significantly reduced inoculum viability (N90 %) after 24 hr of incubation. The standard broth dilution methods determined the minimum inhibitory concentration of *S. asoca* extracts on fish pathogens of *O. niloticus* (CLSI M07-A9). The plant extract was serially diluted two-fold in concentrations in sterile MHB in sterile test tubes ranging from 250 mg/mL to 1.95 mg/mL, with adjusted bacterial concentration (10^8 CFU/mL, 0.5 McFarlands' standard) to calculate MIC. Bacterial inoculum was used as a positive control and MHB was set as blank. Growth was determined by measuring the optical density of each sample at 600 nm.

Statistical analysis

The significant difference ($p<0.05$) was determined by one-way ANOVA operating DMRT in IBM SPSS statistics 21. All the values were expressed as mean ± SD (n=3).

Results

GC MS spectral analysis

The GC-MS study of the methanol fraction of *S. asoca* leaves found six chemicals exhibiting a range of phytochemical activity. The chromatogram is shown in Fig. 2 and the chemical compounds with retention time (RT), molecular weight (g/mol) and peak area (%) are presented in Table 1. The GC-MS study performed on the methanol fraction of *S. asoca* revealed the presence of the following bioactive compounds: 3- hydroxy biphenyl, n-hexa decanoic acid, oleic acid, octadecanoic acid, 4,5-diethyl octane, 9-tetradecen-1-ol.

FT-IR spectral analysis

FTIR spectra give the ranges of absorptions and functional groups like hydroxyl (O-H) at 3738.61 cm^{-1}, amine (N-H) at 2927.02 cm^{-1}, alkane (C-H) at 2848. 25 cm^{-1}, nitro compounds (N-O) at 1518.67 cm^{-1}, vinyl ether (C-O) at 1227.45 cm^{-1}, ketone (C=C) at 1616.63 cm^{-1}, alkene (C=C=C) at 2771.81 cm^{-1}, acid halide (C=O) at 1802.55 cm^{-1}, carboxylic acid (O-H) at 1442.28 cm^{-1}, aromatic ester (C-O) at 1315.77 cm^{-1}, vinyl ether (C-O) at 1227.45 cm^{-1}, alkyl aryl ether (C=C) at 1022.18 cm^{-1} in the plant sample are depicted in Table 2 and Fig. 3.

Table 1. Bioactive compounds found in the methanol fraction of *S. asoca*

Sl. No.	RT (min)	Name of the compound	Molecular formula	Molecular weight (g/mol)	Peak area %
1.	17.568	3- hydroxy biphenyl	$C_{12}H_{10}O$	170.207	24.25
2.	19.476	n-hexa decanoic acid	$C_{16}H_{32}O_2$	256.424	36.46
3.	20.600	Oleic acid	$C_{18}H_{34}O_2$	282.461	18.16
4.	20.681	Octadecanoic acid	$C_{18}H_{36}O_2$	284.477	11.32
5.	20.916	4,5-diethyl octane	$C_{12}H_{26}$	170.334	9.39
6.	22.086	9-Tetradecen-1-ol	$C_{14}H_{28}O$	212.371	0.41

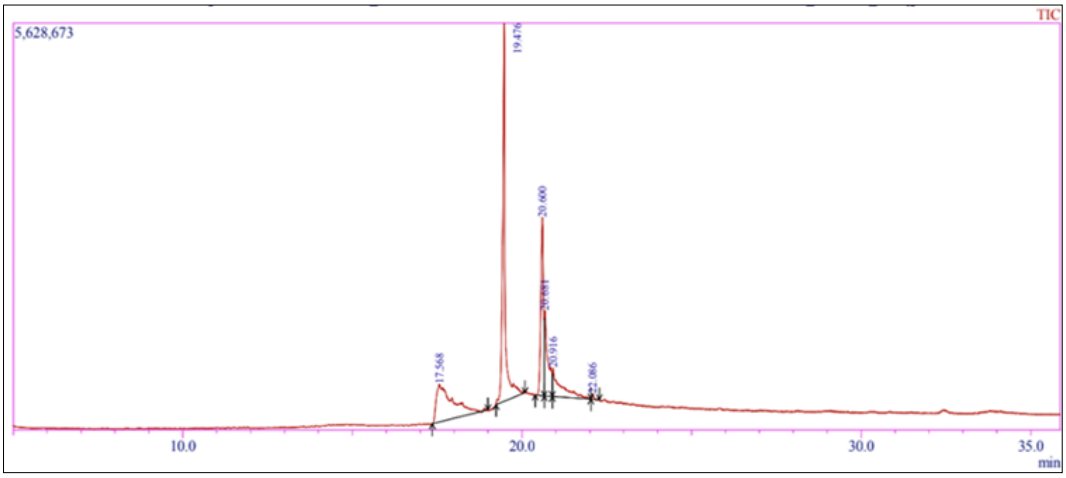

Fig. 2. GC-MS spectrum of *S. asoca* leaves.

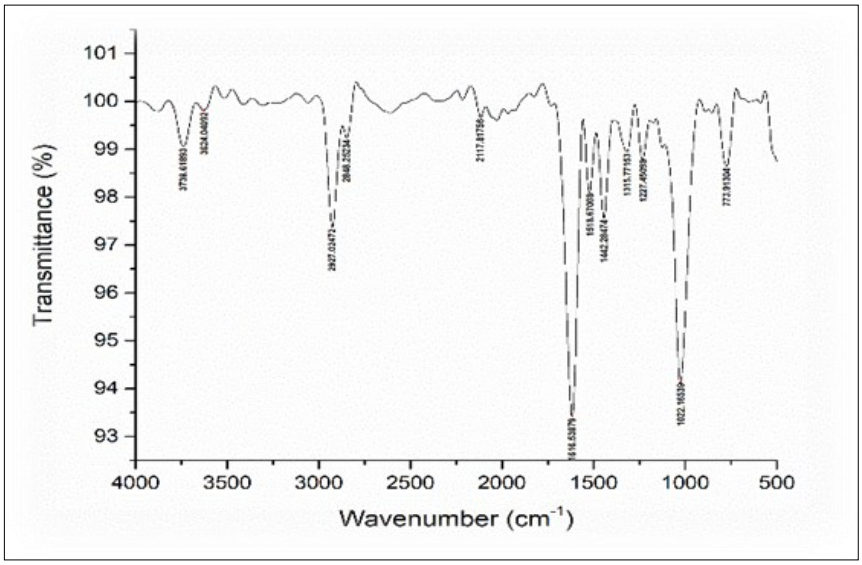

Fig. 3. FT-IR spectrum of *S. asoca* leaves.

Table 2. FTIR spectral analysis of *S. asoca* leaf

Sl. No	Wavenumber range (cm^{-1})	Intensity	Bond	Type of vibration	Functional group assignment
1.	3738.61	medium	O–H	stretch	alcohol
2.	3624.04	medium	O–H	stretch	alcohol
3.	2927.02	strong	N–H	stretch	amine salt
4.	2848.25	weak	C–H	stretch	alkane
5.	2117.81	medium	C=C=C	stretch	alkene
6.	1616.63	strong	C=C	stretch	α,β– unsaturated ketone
7.	1518.67	strong	N–O	stretch	nitro compounds
8.	1442.28	medium	O–H	bending	carboxylic acid
9.	1315.77	strong	C–O	stretch	aromatic ester
10.	1227.45	strong	C–O	stretch	vinyl ether
11.	1022.18	strong	C=C	stretch	alkyl aryl ether
12.	773.91	strong	C–H	bending	monosubstituted

Antibacterial assay : The antibacterial activity of methanol, ethanol and acetone extracts was studied against *V. alginolyticus*, *S. pyrogens*, *P. fluorescens* and *A. hydrophila* and the zone of inhibition (ZI) was measured. These bacterial strains cause infections in the fish species *O. niloticus*. According to the assay data, all three extracts could prevent bacterial development. The growth of the microbes was inhibited in a concentration-dependent manner. The maximum ZI for V. alginolyticus was about 18 ± 0.57 mm for methanolic extract, whereas *S. pyrogens*, *P. fluorescens* and *A. hydrophila* exhibited a zone of clearance of 12 ± 0.23 mm, 14 ± 0.63 mm and 12.3 ± 0.0 mm for methanolic, acetone and ethanol extract respectively which is shown in Fig. 4 and Table 3. There was no effect on the bacterial growth for the negative control (solvent), whereas the positive control (ampicillin) showed clear ZI against *P. fluorescens* and *V. alginolyticus.*

Minimum inhibitory concentration: The concentration effect of *S. asoca* leaf extract was reported in Table 4. The inhibitory effect of *S. asoca* extract varied among the different species of pathogens. The *S. asoca* leaf methanolic extract suppressed the bacterial growth of *V. alginolyticus* at a concentration of 1.95 mg/mL, *S. pyrogens* at 3.91 mg/mL, *P. fluorescens* at a concentration of 3.91 mg/mL and *A. hydrophila* at a concentration of 1.95 mg/mL.

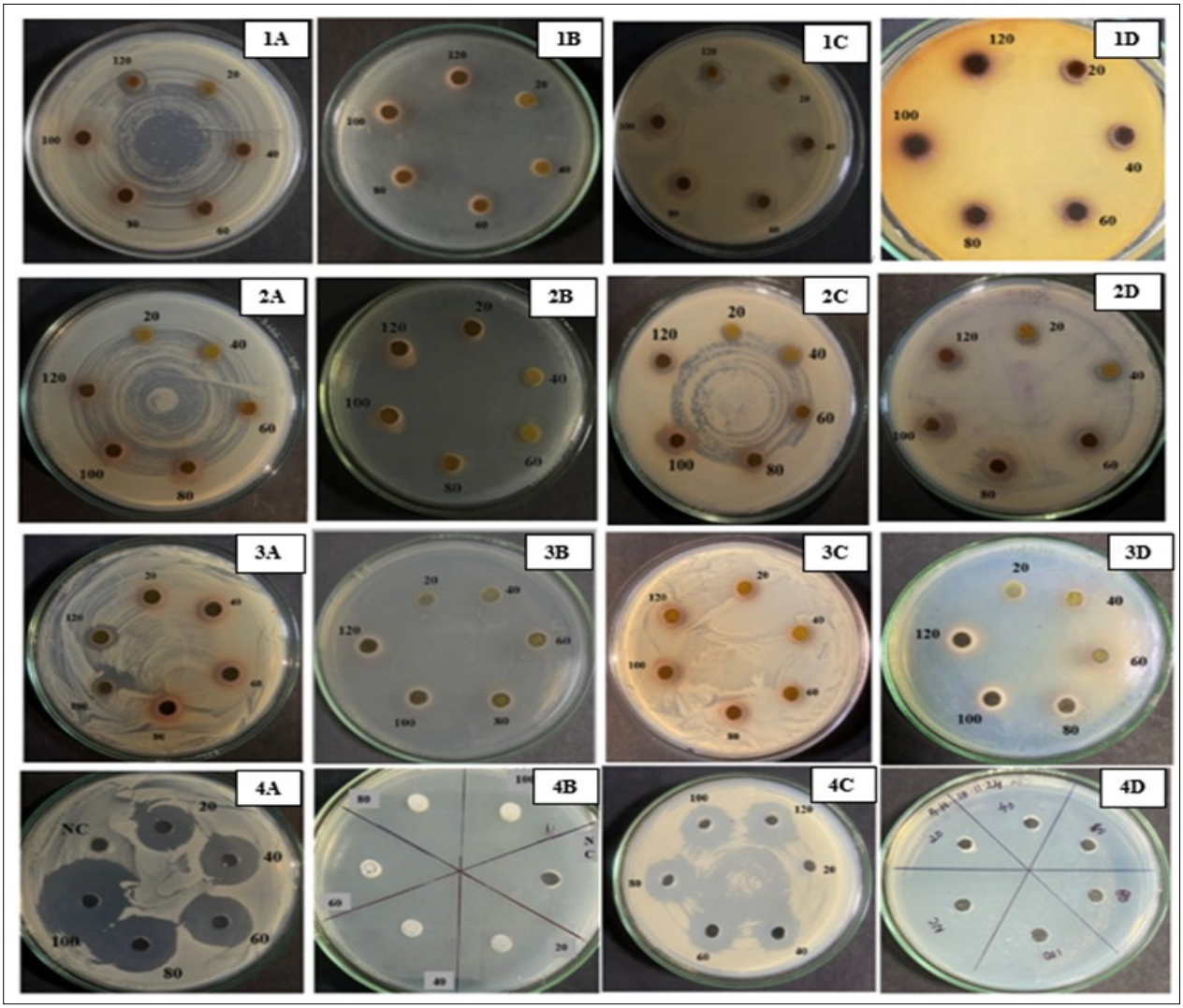

Fig. 4. Antibacterial activity of various extracts of *S. asoca* against pathogens of *O. niloticus*. i. (A-D) *P. fluorescens*, *S. pyrogens*, *V. alginolyticus* and *A. hydrophila*; ii. (1-3) indicates the methanolic, ethanolic and acetone extracts of *S. asoca*; iii. (4) indicates negative control and positive control.

Table 3. Measure the zone of inhibition among different extracts of *S. asoca* against bacterial strains infecting *O. niloticus*

Plant extracts	Concentration (mg/mL)	Tested bacterial strains (zone of inhibition in mm)			
		V. alginolyticus	*S. pyrogens*	*P. fluorescens*	*A. hydrophila*
Methanol	20	$10^{a} \pm 0.23$	$6^{c} \pm 0.21$	$6.2^{c} \pm 0.15$	$8^{b} \pm 0.42$
	40	$12^{a} \pm 1.32$	$6.5^{d} \pm 0.6$	$7.1^{c} \pm 0.65$	$8.5^{b} \pm 0.63$
	60	$14^{a} \pm 0.65$	$7^{d} \pm 0.2$	$8^{c} \pm 0.25$	$9^{b} \pm 0.51$
	80	$15^{a} \pm 1.31$	$8^{d} \pm 0.35$	$9^{c} \pm 0.32$	$10^{b} \pm 0.12$
	100	$15.5^{a} \pm 0.68$	$11^{b} \pm 1.2$	$9.7^{c} \pm 0.56$	$11^{b} \pm 1.56$
	120	$18^{a} \pm 0.57$	$12^{b} \pm 0.23$	$12^{b} \pm 0.56$	$12^{b} \pm 0.78$
Ethanol	20	$8^{a} \pm 1.23$	$6^{c} \pm 0.02$	$7^{b} \pm 0.25$	$8.2^{a} \pm 0.25$
	40	$9^{b} \pm 0.69$	$7^{d} \pm 0.36$	$8^{c} \pm 0.56$	$10^{a} \pm 0.69$
	60	$10^{b} \pm 0.0$	$7.3^{d} \pm 0.5$	$8.2^{c} \pm 0.25$	$11^{a} \pm 0.58$
	80	$11^{a} \pm 0.84$	$8^{c} \pm 0.52$	$10^{b} \pm 0.0$	$11.5^{a} \pm 0.14$
	100	$13^{a} \pm 0.63$	$8.7^{d} \pm 0.1$	$10.1^{c} \pm 0.85$	$12^{b} \pm 1.20$
	120	$14^{a} \pm 2.1$	$9^{d} \pm 0.0$	$11^{c} \pm 0.25$	$12.3^{b} \pm 0.0$
Acetone	20	$7^{a} \pm 0.35$	$7^{a} \pm 0.23$	$7^{a} \pm 1.0$	$7^{a} \pm 1.63$
	40	$8^{a} \pm 0.24$	$8^{a} \pm 0.85$	$8^{a} \pm 0.0$	$8^{a} \pm 1.32$
	60	$9^{b} \pm 0.78$	$9^{b} \pm 0.59$	$10^{a} \pm 1.3$	$9^{b} \pm 8.2$
	80	$10^{b} \pm 0.26$	$10^{b} \pm 1.05$	$12^{a} \pm 0.31$	$10^{b} \pm 0.0$
	100	$11^{b} \pm 0.69$	$10^{c} \pm 0.63$	$13^{a} \pm 0.25$	$11^{b} \pm 0.82$
	120	$12^{b} \pm 0.35$	$11^{c} \pm 0.25$	$14^{a} \pm 0.63$	$12^{b} \pm 0.75$
Ampicillin (standard)	20	15 ± 0.1	–	18 ± 0.87	–
	40	16 ± 0.23	–	19 ± 0.06	–
	60	17 ± 0.45	–	22 ± 0.39	–
	80	20 ± 0.58	–	24 ± 0.0	–
	100	21 ± 1.0	–	30 ± 0.09	–
Negative control (methanol/ethanol/ acetone)	–	–	–	–	–

All the results are expressed as mean ± SD (n = 5), '–': no zone of inhibition, the same letters indicate no significant difference whereas different letters indicate significant difference at a 5 % probability level

Table 4. Minimum inhibitory concentration (MIC; mg/mL) for *S. asoca* extract against pathogenic bacteria of *O. niloticus*

Bacterial Culture	Minimum inhibitory concentration (mg/mL)
V. alginolyticus	1.95
S. pyrogens	3.91
P. fluorescens	3.91
A. hydrophila	1.95

Discussion

The current study aimed to validate the phytochemicals contained in plant extracts and assess their antibacterial activity against pathogens of *O. niloticus*. 80 percentage of the worlds' population depends on traditional medicine for basic health care, a system of medicine in which most drugs and treatments are derived from plants (30). It has been reported that plants are used in healthcare mainly due to the bioactive molecules with therapeutic capabilities, such as antibacterial, anti-inflammatory and analgesic actions and they frequently have fewer side effects than manufactured medications (31). In addition, plant-based therapies are affordable, sustainable and culturally significant possibilities for controlling chronic illnesses (32), improving general health and acting as potential sources for novel drug development (33). Plant extracts play a vital role in aquaculture systems; they are often included in the diet to improve immunity, growth and development and resistance against pathogens in fish species (34). Studies showed that *Nigella sativa* can enhance overall immunity and reduce vulnerability to *Vibrio harveyi* in gilthead sea bream (35). Another study revealed that *Andrographis paniculata* effectively prevents *A. hydrophila* infections in aquaculture, ensuring fish health (36). Researchers also reported that extracts from plants like garlic (37), ginger (38) and seaweed (39) contain bioactive compounds with antibacterial, anti-inflammatory and immune-boosting properties, enhancing fish health and infection resilience.

The GC-MS study of methanol extracts of *S. asoca* yielded many substances with various characteristics. The constituent 3-hydroxy biphenyl consists of biphenyl with one hydroxyl group (-OH) attached to one of the rings and it has been reported that hydroxy biphenyl acts as a ligand for metal ion complexes in wastewater treatment (40). The constituent hexadecanoic acid is a saturated fatty acid found in animal and protein fats with antibacterial effects. Studies showed that hexadecanoic acid has antibacterial effects, specifically against gram-positive bacteria like *Staphylococcus aureus* (41). It has also been demonstrated that hexadecenoic acid methyl esters from the clove alcoholic extract and silver nanoparticles from the clove were responsible for antibacterial activity against multidrug -resistant bacteria (42). This might be due to the fatty acids' ability to disrupt the integrity of bacterial cell membranes (43). The component oleic acid has antibacterial and antifungal properties. Researchers showed that *Rosa damascena* Mill has a strong effect against gram-negative bacterium *Klebsiella pneumonia* and *Aspergillus brasiliensis* (44). GC-MS analysis of the methanolic fraction of *S. asoca* also showed the presence of octadecanoic acid, also known as stearic acid. It was shown that the stearic acid improves the dispersion of (Tetra-Needle-like zinc oxide whiskers) T-ZnOw in the rubber matrix, enhancing composite performance and slightly boosting the antibacterial effect by optimizing the interfacial bonding between natural rubber and T-ZnOw (45). 9-Tetradecen-1-ol, an unsaturated fatty alcohol used in pheromones, fragrances and cosmetics, was also detected in *S. asoca* extract (46).

In the FT-IR spectral analysis, several prominent peaks indicated the presence of specific functional groups in the *S. asoca* leaf fraction. The result showed high absorbance at the wavenumber region of 4000-3500, 3000-2500, 1800-1500 and 1100-950 cm^{-1}. Hydroxy can create hydrogen bonds with proteins, nucleic acids and bacterial cell membrane components. This bonding can interfere with the operation of bacterial enzymes or cell surface proteins, limiting their ability to execute critical functions such as food absorption, membrane integrity and DNA replication (47). The graph shows a prominent stretch at the wavenumber 2927.02 cm^{-1}, signifying amine salt. The cationic nature of the amine group allows it to interact with the negatively charged cell membranes of bacteria, resulting in the disruption of cell integrity and, eventually, cellular components leakage, ultimately killing the bacteria (48). The extract showed medium-intensity absorption at 3293.3 cm^{-1} because of C=C=C stretching and its compound classs' alkene. Double bonds in alkene are essential in their reactivity and can contribute to antibacterial action via membrane disruption, enzyme inhibition, free radical production and biofilm interference (49). The extract displayed a medium absorption peak at 1616.63 cm^{-1} because of C=C stretching indicative of unsaturated ketones.

Curcumin in turmeric has an unsaturated structure and a carbonyl group that showed antibacterial activity, notably against gram-positive bacteria, via mechanisms such as membrane rupture and enzyme inhibition (50). An intense absorption peak at 1518.67 cm^{-1} due to N-O stretching vibrations and its compound classs' group nitro compounds. Nitroimidazoles are examples of nitro compounds with antibacterial effects, especially against gram-positive, gram-negative and anaerobic bacteria (51). A medium O-H bending at 1442.28 cm^{-1}, indicating carboxylic acid, was also determined. One of the carboxylic acids, propionic acid supplementation, enhances the antibacterial and immune modulatory effect in *O. niloticus* (52). There was also a strong C-O stretching at 1315.77 cm^{-1}, indicating aromatic esters, 1227.45 cm^{-1}, indicating vinyl ether and C=C stretching at 1022.18 cm^{-1}, showing the presence of alkyl aryl ether. Wintergreen oil contains methyl salicylate, an aromatic ester with antibacterial effects against many microorganisms, including gram-positive and gram-negative (53). Vinyl ether-based polymers and copolymers have been found to have antibacterial characteristics. These polymers are commonly utilized in biomedical equipment, wound dressings and surface coatings (54). Anisole (methoxybenzene) is an aryl alkyl ether found in natural

products and synthetic drugs (55). Lastly, it revealed a strong absorption at 773.91 cm^{-1} due to C-H bending, corresponding to monosubstituted compounds. These monosubstituted compounds can exhibit a wide range of biological activities, including antibacterial properties that depend on the nature of the substituent, its position on the parent structure and the overall chemical properties of the compound (56).

The antibacterial activity of plant extracts depends significantly on the solvent used for extraction (57). The current study employed three distinct extracts, including methanol, ethanol and acetone extract of *S. asoca*, for the antibacterial test against bacterial pathogens of *O. niloticus*. The findings indicated that acetone and methanol extracts had the most substantial inhibitory zones (18 ± 0.57 and 12 ± 0.35 mm, respectively) against *V. alginolyticus*. The acetone extracts inhibited *S. pyrogens* more effectively than ampicillin, with an inhibition zone of 11 ± 0.25mm. This value is greater than the inhibition zones induced by ethanol extracts, with methanol having the most significant inhibition zone of 12 ± 0.23. There was no zone of clearance by ampicillin against *S. pyrogens*.

On the other hand, acetone extracts with a zone of 14 ± 0.63 were more active towards *P. fluorescens* than methanol extract and ethanol extract. Our extracts demonstrated activity against *P. fluorescens*, which was less than 47 % of the efficacy of ampicillin. Polar solvents, such as acetone, are more effective for extracting lipophilic compounds, such as essential oils and terpenoids, which have potent antimicrobial properties (58). Methanol, acetone and ethanol extracts inhibited gram-negative and gram-positive bacterial strains. Ethanolic and methanolic extracts typically show the most potent antibacterial activity due to their ability to extract a wide range of bioactive compounds, including flavonoids, alkaloids and essential oils (59).

Furthermore, the capability of ethanol extract to inhibit *A. hydrophila* with a ZI of 12.3 ± 0. The choice of solvent thus determines the spectrum and strength of antibacterial activity, making solvent selection a critical factor when developing plant-based antibacterial products (60). Therefore, this study demonstrates that the antibacterial capabilities of *S. asoca* are primarily due to the functional groups such as carboxyl, hydroxy and amino present in them, which have been shown to exhibit antibacterial activity against a wide range of pathogens, including gram-positive and gram-negative bacteria. These bioactive chemicals exhibit antimicrobial actions by disrupting cell membranes, reducing enzyme activity and interfering with bacterial protein production (61). In addition, chemicals with antimicrobial, antioxidant and anti -inflammatory characteristics can activate fishs' immune systems, reduce stress and function as antibacterial and antiparasitic agents, safeguarding them (62).

Conclusion

Bioactive compounds delivered through plant extracts or whole plant material have intriguing therapeutic potential in aquaculture. However, efficacy is impacted by parameters such as plant portion utilized, location and harvest season. *S. asoca*, a plant with well-established therapeutic properties, particularly as a female tonic, contains bioactive compounds that exhibit anti-inflammatory, antibacterial, anticarcinogenic, free radical-scavenging and hypolipidemic effects. It is a vast research opportunity to explore its potential as an antibacterial agent against fish infections, particularly those that impact species such as *O. niloticus*. The current findings of the study show that *S. asoca* has the potential to improve aquaculture system health and production while lowering reliance on synthetic chemicals and increasing environmental sustainability in Indias' expanding aquaculture sector. However, issues such as active chemical concentration fluctuation must be addressed. Plant extracts must be rigorously tested and standardized to assure their safety, effectiveness and consistency, thereby minimizing antibiotic resistance in aquaculture and extending their medical uses.

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Authors' contributions

MS and MP wrote the study's conceptualization, and the original draft of the manuscript, guided and edited the work. All authors carefully read and approved the final manuscript.

Compliance with ethical standards

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References

1. The State of World Fisheries and Aquaculture 2020 [Internet]. Rome: FAO; 2020 [cited 2024 Dec 9]. Available from: https://doi.org/10.4060/ca9229en
2. The State of World Fisheries and Aquaculture 2022 [Internet]. Rome: FAO; 2022 [cited 2024 Dec 13]. Available from: https://doi.org/10.4060/cc0461en
3. Zhang J, Yang X, Wang Z, Liu Y, Liu X, Ding Y. Mapping of land-based aquaculture regions in Southeast Asia and its Spatiotemporal change from 1990 to 2020 using time-series remote sensing data. Int J Appl Earth Obs Geoinf. 2023;124:103518. https://doi.org/10.1016/j.jag.2023.103518
4. The State of Food and Agriculture 2022 [Internet]. Rome: FAO; 2022. [cited 2024 Dec 13].https://doi.org/10.4060/cb9479en
5. Fayaz Mohd, Ahmed M. Fisheries exports of India: A constant market share analysis. Indian Econ J . 2020;68(1):29–39. https://doi.org/10.1177/0019466220959572
6. ICAR-Central Institute of Freshwater Aquaculture, Saha S. Present status of Fish disease management in freshwater aquaculture in

India: state-of-the-art-review. Aquac Fish. 2017;1(1):1–9. http://doi.org/:10.24966/AAF-5523/100003

7. McArthur DB. Emerging infectious diseases. Nurs Clin North Am. 2019;54(2):297–311. https://doi.org/10.1016/j.cnur.2019.02.006
8. Arumugam M, Jayaraman S, Sridhar A, Venkatasamy V, Brown PB, Abdul Kari Z, et al. Recent advances in Tilapia production for sustainable developments in Indian aquaculture and its economic benefits. Fishes. 2023;8(4):176. https://doi.org/10.3390/fishes8040176
9. Haenen OLM, Dong HT, Hoai TD, Crumlish M, Karunasagar I, Barkham T, Chen SL, Zadoks R, Kiermeier A, Wang B, Gamarro EG. Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. Rev Aquac. 2023;15(S1):154–85. https://doi.org/10.1111/raq.12743
10. Manchanayake T, Salleh A, Amal MNA, Yasin ISM, Zamri-Saad M. Pathology and pathogenesis of Vibrio infection in fish: A review. Aquac Rep. 2023;28:101459. https://doi.org/10.1016/j.aqrep.2022.101459
11. Liao PC, Tsai YL, Chen YC, Wang PC, Liu SC, Chen SC. Analysis of Streptococcal infection and correlation with climatic factors in cultured Tilapia Oreochromis spp. in Taiwan. Appl Sci. 2020;10 (11):4018. https://doi.org/10.3390/app10114018
12. Shabana BM, Elkenany RM, Younis G. Sequencing and multiple antimicrobial resistance of Pseudomonas fluorescens isolated from Nile tilapia fish in Egypt. Braz J Biol. 2024;84:e257144. https://doi.org/10.1590/1519-6984.257144
13. Heeb S, Fletcher MP, Chhabra SR, Diggle SP, Williams P, Cámara M. Quinolones: from antibiotics to autoinducers. FEMS Microbiol Rev. 2011;35(2):247–74. https://doi.org/10.1111/j.1574-6976.2010.00247.x
14. Vignesh R, Karthikeyan BS, Periyasamy N, Devanathan K. Antibiotics in Aquaculture: An Overview. South Asian J Exp Biol. 2011;1(3):114–20. https://doi.org/10.38150/sajeb.1(3).p114-120
15. Chuah LO, Effarizah ME, Goni AM, Rusul G. Antibiotic application and emergence of Multiple Antibiotic Resistance (MAR) in global catfish aquaculture. Curr Environ Health Rep. 2016;3(2):118–27. https://doi.org/10.1007/s40572-016-0091-2
16. Radhakrishnan A, Vaseeharan B, Ramasamy P, Jeyachandran S. Oral vaccination for sustainable disease prevention in aquaculture-an encapsulation approach. Aquac Int. 2023 31 (2):867–91. https://doi.org/10.1007/s10499-022-01004-4
17. García M. Debating diseases in nineteenth-century Colombia: Causes, interests and the pasteurian therapeutics. Bull Hist Med. 2015;89(2):293–321. https://dx.doi.org/10.1353/bhm.2015.0050
18. Tammas I, Bitchava K, Gelasakis AI. Transforming aquaculture through vaccination: A review on recent developments and milestones. Vaccines. 2024;12(7):732. https://doi.org/10.3390/vaccines12070732
19. Yu N tong, Zeng W wei, Xiong Z, Liu Z xin. A high efficacy DNA vaccine against Tilapia lake virus in Nile tilapia (*Oreochromis niloticus*). Aquac Rep. 2022;24:101166. https://doi.org/10.1016/j.aqrep.2022.101166
20. Su H, Yakovlev IA, Van Eerde A, Su J, Clarke JL. Plant-produced vaccines: Future applications in aquaculture. Front Plant Sci. 2021;12:718775. https://doi.org/10.3389/fpls.2021.718775
21. Kuebutornye FKA, Abarike ED. The contribution of medicinal plants to tilapia aquaculture: a review. Aquac Int. 2020;28(3):965–83. https://doi.org/10.1007/s10499-020-00506-3
22. Leyva-López N, Lizárraga-Velázquez CE, Hernández C, Sánchez-Gutiérrez EY. Exploitation of agro-industrial waste as a potential source of bioactive compounds for aquaculture. Foods. 2020;9 (7):843. https://doi.org/10.3390/foods9070843
23. Brusotti G, Cesari I, Dentamaro A, Caccialanza G, Massolini G. Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. 2014;87:218–28 http://doi.org/10.1016/j.jpba.2013.03.007
24. Dawood MAO, El Basuini MF, Zaineldin AI, Yilmaz S, Hasan MdT, Ahmadifar E,et al. Antiparasitic and antibacterial functionality of essential oils: An alternative approach for sustainable aquaculture. Pathogens. 2021;10(2):185. https://doi.org/10.3390/pathogens10020185
25. Prasad S, Younis K, Yousuf O. Investigating potent cardioprotective compounds as ACE inhibitors in *Saraca asoca*. Toxicol Rep. 2024;13:101731. https://doi.org/10.1016/j.toxrep.2024.101731
26. Suresh M, Pappuswamy M. *Saraca asoca* (Roxb.) de Klilde, a sacred tree: its nutritional value, elemental composition and anti-nutritional content. Plant Sci Today. 2024;11(4). https://doi.org/10.14719/pst.3924
27. Nigussie D, Davey G, Legesse BA, Fekadu A, Makonnen E. Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients. BMC Complement Med Ther. 2021;21 (1):2. https://doi.org/10.1186/s12906-020-03183-0
28. Ekom SE, Tamokou JDD, Kuete V. Antibacterial and therapeutic potentials of the Capsicum annuum extract against infected wound in a rat model with its mechanisms of antibacterial action. Formanowicz D, editor. BioMed Res Int. 2021;2021:1–17. https://doi.org/10.1155/2021/4303902
29. Liaqat N, Jahan N, Khalil-ur-Rahman, Anwar T, Qureshi H. Green synthesized silver nanoparticles: Optimization, characterization, antimicrobial activity and cytotoxicity study by hemolysis assay. Front Chem. 2022;10:952006. https://doi.org/10.3389/fchem.2022.952006
30. Manke MB, Dhawale SC, Jamkhande PG. Helminthiasis and medicinal plants: a review. Asian Pac J Trop Dis. 2015;5(3):175–80. https://doi.org/10.1016/S2222-1808(14)60648-4
31. Christaki E, Bonos E, Giannenas I, Florou-Paneri P. Aromatic plants as a source of bioactive compounds. Agric. 2012 ;2(3):228–43. https://doi.org/10.3390/agriculture2030228
32. Hever J, Cronise RJ. Plant-based nutrition for healthcare professionals: implementing diet as a primary modality in the prevention and treatment of chronic disease. J Geriatr Cardiol. 2017;14(5):355–68. http://doi.org/10.11909/j.issn.1671-5411.2017.05.012
33. Najmi A, Javed SA, Al Bratty M, Alhazmi HA. Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. Molecules. 2022;27(2):349. https://doi.org/10.3390/molecules27020349
34. Reverter M, Tapissier-Bontemps N, Sarter S, Sasal P, Caruso D. Moving towards more sustainable aquaculture practices: a meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. Rev Aquac. 2021;13 (1):537–55. https://doi.org/10.1111/raq.12485
35. Aly SM, ElBanna NI, Elatta MA, Hegazy M, Fathi M. Effects of natural and synthetic immunostimulants on growth, feed utilization, immune status and disease resistance against vibriosis in sea bream (*Sparus aurata*). Aquac Int. 2024;32(3):2739–56. https://doi.org/10.1007/s10499-023-01294-2
36. Palanikani R, Chanthini KMP, Soranam R, Thanigaivel A, Karthi S, Senthil-Nathan S, et al. Efficacy of *Andrographis paniculata* supplements induce a non-specific immune system against the pathogenicity of Aeromonas hydrophila infection in Indian major carp (*Labeo rohita*). Environ Sci Pollut Res. 2020;27 (19):23420–36. https://doi.org/10.1007/s11356-019-05957-7
37. Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H, et al. Bioactive compounds and biological functions of garlic (*Allium sativum* L.). Foods. 2019;8(7):246. https://doi.org/10.3390/foods8070246
38. Rawat P, Kaur VI, Tyagi A, Norouzitallab P, Baruah K. Determining the efficacy of ginger Zingiber officinale as a potential nutraceutical agent for boosting growth performance and health status of *Labeo rohita* reared in a semi-intensive culture system.

Front Physiol. 2022;13:960897. https://doi.org/10.3389/fphys.2022.960897

39. Siddik MAB, Francis P, Rohani MF, Azam MS, Mock TS, Francis DS. Seaweed and seaweed-based functional metabolites as potential modulators of growth, immune and antioxidant responses and gut microbiota in fish. Antioxidants. 2023;12(12):2066. https://doi.org/10.3390/antiox12122066

40. Awad AAA, Moustafa Ali OA, Fattah Nassar DA. Degradation of dye wastewater by using cobalt, copper and nickel Schiff base complexes as catalysts: spectral, molecular modelling, catalytic activity and metal removal from aqueous solution. Int J Environ Anal Chem. 2023;103(20):9562–81. https://doi.org/10.1080/03067319.2021.2014466

41. Sanabria-Ríos DJ, Morales-Guzmán C, Mooney J, Medina S, Pereles-De-León T, Rivera-Román A, et al. Antibacterial activity of hexadecynoic acid isomers toward clinical isolates of multidrug-resistant *Staphylococcus aureus*. Lipids. 2020;55(2):101–16. https://doi.org/10.1002/lipd.12213

42. Shaaban MT, Ghaly MF, Fahmi SM. Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. J Basic Microbiol . 2021;61(6):557–68. https://doi.org/10.1002/jobm.202100061

43. Yuyama KT, Rohde M, Molinari G, Stadler M, Abraham WR. Unsaturated fatty acids control biofilm formation of Staphylococcus aureus and other gram-positive bacteria. antibiotics. 2020;9(11):788. https://doi.org/10.3390/antibiotics9110788

44. Ghavam M, Afzali A, Manca ML. Chemotype of damask rose with oleic acid (9 octadecenoic acid) and its antimicrobial effectiveness. Sci Rep. 2021;11(1):8027. https://doi.org/10.1038/s41598-021-87604-1

45. Chen X, Wang Z, Wu J. Processing and characterization of natural rubber/stearic acid-tetra-needle-like zinc oxide whiskers medical antibacterial composites. J Polym Res . 2018;25(2):48. https://doi.org/10.1007/s10965-017-1433-y

46. Han S, He K, An J, Qiao M, Ke R, Wang X, et al. Detection of specific volatile organic compounds in Tribolium castaneum (Herbst) by solid-phase microextraction and gas chromatography-mass spectrometry. Foods. 2023;12(13):2484. https://doi.org/10.3390/foods12132484

47. Chen J, Yang J, Ma L, Li J, Shahzad N, Kim CK. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl and carboxylic acid groups of phenolic acids. Sci Rep.. 2020;10(1):2611. https://doi.org/10.1038/s41598-020-59451-z

48. Qian Y, Deng S, Cong Z, Zhang H, Lu Z, Shao N, et al. Secondary amine pendant β-peptide polymers display potent antibacterial activity and promising therapeutic potential in treating MRSA-induced wound infections and Keratitis. J Am Chem Sock 2022;144(4):1690–9. https://doi.org/10.1021/jacs.1c10659

49. Burman S, Bhattacharya K, Mukherjee D, Chandra G. Antibacterial efficacy of leaf extracts of Combretum album Pers. against some pathogenic bacteria. BMC Complement Altern Med. 2018;18(1):213. https://doi.org/10.1186/s12906-018-2271-0

50. Dai C, Lin J, Li H, Shen Z, Wang Y, Velkov T, et al. The natural product curcumin as an antibacterial agent: Current achievements and problems. Antioxidants. 2022;11(3):459. https://doi.org/10.3390/antiox11030459

51. Reinhardt T, Lee KM, Niederegger L, Hess CR, Sieber SA. Indolin-2-one Nitroimidazole Antibiotics Exhibit an Unexpected Dual Mode of Action. ACS Chem Biol. 2022;17(11):3077–85. https://doi.org/10.1021/acschembio.2c00462

52. El-Adawy M, El-Aziz MA, El-Shazly K, Ali NG, El-Magd MA. Dietary propionic acid enhances antibacterial and immunomodulatory effects of oxytetracycline on Nile tilapia, *Oreochromis niloticus*. Environ Sci Pollut Res. 2018;25(34):34200–11. https://doi.org/10.1007/s11356-018-3206-5

53. Michel P, Olszewska MA. Phytochemistry and biological profile of *Gaultheria procumbens* L. and wintergreen essential oil: From traditional application to molecular mechanisms and therapeutic targets. Int J Mol Sci . 2024 Jan;25(1): 565https://doi.org/10.3390/ijms25010565

54. Ergene C, Yasuhara K, Palermo EF. Biomimetic antimicrobial polymers: recent advances in molecular design. Polym Chem. 2018;9(18):2407–27. https://doi.org/10.1039/C8PY00012C

55. Vani B, Pabba M, Kalyani S, Sridhar S. Separation of anisole and valuable byproducts from liquid reaction mixtures by solvent extraction and multicomponent distillation. J Solut Chem. 2021;50(1):160–77. https://doi.org/10.1007/s10953-020-01049-0

56. Yuan H, Ou H, Cao C. The application of a new approach based on organic homo-rank compounds and homologous compounds to the structure-property relationship study of monosubstituted alkanes. J Phys Org Chem. 2016;29(1):42–58. https://doi.org/10.1002/poc.3486

57. Ibrahim N, Kebede A. *In vitro* antibacterial activities of methanol and aqueous leaf extracts of selected medicinal plants against human pathogenic bacteria. Saudi J Biol Sci . 2020;27(9):2261–8. https://doi.org/10.1016/j.sjbs.2020.06.047

58. Saleh-E-In MdM, Bhattacharyya P, Van Staden J. Chemical composition and cytotoxic activity of the essential oil and oleoresins of in vitro micropropagated Ansellia africana Lindl: A vulnerable medicinal orchid of Africa. Mol. 2021;26(15):4556. https://doi.org/10.3390/molecules26154556

59. Ngo TV, Scarlett CJ, Bowyer MC, Ngo PD, Vuong QV. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis* L. J Food Qual. 2017;2017:1–8. https://doi.org/10.1155/2017/9305047

60. Sibi G, Naveen R, Dhananjaya K, Ravikumar KR, Mallesha H. Potential use of Muntingia calabura L. extracts against human and plant pathogens. Pharmacogn J . 2012;4(34):44–7. https://doi.org/10.5530/pj.2012.34.8

61. Unuofin JO, Lebelo SL. Antioxidant effects and mechanisms of medicinal plants and their bioactive compounds for the prevention and treatment of type 2 diabetes: An updated review. Oxid Med Cell Longev. 2020;2020:1–36. http://doi.org/10.1155/2020/1356893

62. Anilkumar AT, Manoharan S, Balasubramanian S, Perumal E. *Garcinia gummi-gutta*: Phytochemicals and pharmacological applications. BioFactors. 2023;49(3):584–99. https://doi.org/10.1002/biof.1943

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