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Comparative assessment of *Rhazya stricta* and *Azadirachta indica* methanolic leaf extracts for their antimicrobial efficacy against the selected foodborne pathogens

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Abstract

To address the rising challenge of resistance to traditional antibiotics and to bolster microbial management in the dominions of food safety and antibiotic effectiveness, recent explorations have been conducted into the bioactive phytochemicals, utilizing eco-friendly methodologies. This study focuses on the potential utility of *Rhazya stricta* and *Azadirachta indica*, indigenous plant species of Saudi Arabia, in combatting foodborne bacteria across the pharmaceutical and food sectors. The dried leaf powders of both plants underwent methanol extraction, evaporation via a rotary evaporator, and subsequent dissolving in a 1% dimethyl sulfoxide (DMSO) solution. The bioactive constituents were analyzed through gas chromatography-mass spectrometry (GC/MS), revealing a higher presence of bioactive compounds (32 compounds) in *A. indica* compared to *R. stricta* (15 compounds). *R. stricta's* methanolic extract demonstrated superior efficacy against the tested bacterial strains (*Salmonella enteritidis, Staphylococcus aureus, Salmonella typhimurium,* and *Escherichia coli*) compared to *A. indica*. These findings were compared against six antibiotics: cefoxitin (FOX), cephalothin (KF), cotrimoxazole (TS), gentamicin (GM), augmentin (AUG), and ampicillin (AP). Furthermore, both plant extracts exhibited inhibitory effects on microbial lipase, amylase, and protease enzymes. In conclusion, further investigations at the molecular and biochemical levels are warranted in future studies to elucidate the precise mechanisms underlying the antibacterial efficacy of these naturally occurring plant species.

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Introduction

In the past two decades, there has been a resurgence of bacterial resistance to antibiotics, a concern that initially emerged in the 1960s. This global issue has prompted the World Health Organization (WHO) and other health organizations to prioritize the battle against human microbial pathogens, particularly those acquired in healthcare settings, and to prioritize the development of new drugs for their treatment. Given that these bacteria exhibit resistance to multiple conventional medications, it is crucial to explore the therapeutic potential of various medicinal and herbal plants (Beigomi *et al.,* 2021).

The study of medicinal plants for the discovery of new treatment modalities, characterized by reduced side effects and enhanced economic value, has gained significant importance worldwide (Tnah *et al.,* 2019; Aware *et al.,* 2022; Jităreanu *et al.,* 2023). Current statistics indicate that herbal medicines are being utilized in hospitals and clinics at a rate exceeding 30% (Gajula and Nanjappan, 2021; Mukne *et al.,* 2022). While antibiotics are invaluable for treating numerous human diseases, their excessive use contributes to the emergence of microbial resistance.

Consequently, scientists have directed their research efforts toward various components of medicinal plants to identify novel plant-based drugs (Pan *et al.,* 2020; Xiao *et al.,* 2022). In the local setting, the use of medicinal plants to treat foodborne illnesses has grown significantly because of two main reasons: a) Saudi Arabia has a huge variety of plants, but we still don't know much about how they are used in the region; and b) Saudi Arabia's 2030 vision includes a shared interest in exploring and exploiting medicinal plants for a variety of purposes (Alharbi, 2017).

Rhazya stricta (Decne.), a member of the subfamily Rauwolfioideae in the family Apocynaceae, is geographically distributed across Iran, Afghanistan, Pakistan, India, Iraq, Oman, Yemen, and Saudi Arabia (Shaer, 2019). In Saudi Arabia, it is commonly known by its local names, "Espand" or "Harmal." Harmal has been traditionally used in the Middle Eastern region for its medicinal properties in the treatment of various ailments (Fazeli-Nasab *et al.,* 2021). *R. stricta* is a small perennial shrub characterized by its toxic nature, persistent foliage, small size, and upright growth habit, with smooth, non-hairy leaves (Ribeiro-Santos *et al.,* 2018). The seed oil of *R. stricta* is considered a potentially rich source of d-tocopherol, a major form of vitamin E (Shehzad *et al.,* 2018).

People have used *R. stricta* and its metabolites to treat a variety of diseases, including cancer, skin disorders, hypertension, rheumatism, sore throat, syphilis, and fever (Ullah, 2012). Previous studies have demonstrated that various parts of *R. stricta* contain a diverse array of phytochemical compounds, such as terpenes, alkaloids, and flavonoids (Albeshri *et al.,* 2021).

Hassan *et al.* (2023) investigated the antibacterial efficacy of five solvent extracts, namely aqueous alkaloids, non-aqueous alkaloids, organic alkaloids, organic non-alkaloids, and full aqueous extracts, against a range of drug-resistant pathogens. Their findings indicated that the organic alkaloid extract was the most effective against *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore, the ethanolic extract of *R. stricta* fruit exhibited potent antimicrobial activity (Sultana and Khalid, 2010). Methanol and chloroform extracts of *R. stricta* roots were found to possess antibacterial and antifungal properties against *E. coli*, *Bacillus subtilis*, *S. aureus*, and *Candida albicans* (Bashir *et al.*, 1994). Additionally, the aqueous extract of *R. stricta* demonstrated antimicrobial activity against *S. aureus* (Ghafari *et al.,* 2021).

Azadirachta indica (L.), commonly known as neem, is a tropical evergreen tree native to the Indian subcontinent (Aneesa and Gayathr, 2016). People have highly valued neem for its various benefits, which include its use as an agricultural pesticide and as a remedy for many common human disorders. Initially, *A. indica* garnered interest for its potential as a non-toxic tool for controlling agricultural

infections. Azadirachtin, a prominent constituent of the neem plant, has gained popularity as a biopesticide (Kilani-Morakchi *et al.,* 2021; Wylie and Merrell, 2022). The neem tree has been extensively used in traditional Indian medicine due to its therapeutic properties, including antipyretic, antacid, antiparasitic, antiviral, anti-inflammatory, and antimicrobial activities (Yadav *et al.*, 2023). Various parts of the neem tree, such as the leaves, bark, seeds, and oil, have been utilized for medicinal purposes.

Researchers have extensively studied *A. indica*'s antimicrobial activity. The leaf extract of *A. indica* has shown efficacy against a broad spectrum of bacteria, including *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium* (Ghosh *et al.,* 2024). Neem leaves contain compounds such as nimbin, nimbidin, and quercetin, which exhibit antimicrobial properties (Nagini *et al.,* 2024). The oil extract of *A. indica* has also demonstrated antimicrobial activity against various bacteria and fungi (Aladejana *et al.*, 2024). In addition, oil *A. indica* has been used topically for the treatment of skin infections and has shown potential in combating drug-resistant bacteria (Wylie and Merrell, 2022).

Several studies have investigated the antibacterial potential of *A. indica* against drug-resistant pathogens. For instance, a study conducted by Nagrale and Kamble (2022) evaluated the antibacterial activity of *A. indica* leaf extract against multidrug-resistant strains of *E. coli* and *S. aureus*. The results showed significant inhibition of bacterial growth by *A. indica* extract. Al-Sarraj (2021) reported the antibacterial activity of *A. indica* against drug-resistant strains of *S. aureus* and *K. pneumoniae*. The mechanism of action of *A. indica* antimicrobial activity is multifaceted. It involves disruption of bacterial cell membranes, inhibition of bacterial adhesion, interference with microbial enzymes, and modulation of the host immune response (Sarkar *et al.,* 2016). *A. indica* compounds have also been found to affect bacterial biofilm formation, which is crucial for the survival and persistence of drug-resistant bacteria (Gajula and Nanjappan, 2021).

While various studies have shown antimicrobial activity in *R. stricta* and *A. indica*, further research is necessary to fully understand their potential as alternative treatments for drug-resistant bacterial infections. Clinical trials and comprehensive studies on their safety, efficacy, and optimal dosage are necessary before they can be considered mainstream therapies.

Additionally, it is crucial to promote responsible and sustainable use of medicinal plants to ensure their long-term availability and conservation of biodiversity. We undertook this investigation to explore the potential antimicrobial properties of the methanolic leaf extracts of *R. stricta* and *A. indica* against various clinical strains of Gram-negative and Gram-positive bacteria, including those associated with foodborne illnesses, considering the adverse consequences of prolonged antibiotic use, such as the emergence of antibiotic-resistant bacteria and the ensuing challenges in the clinical management of infections. We will also determine the ability of both plant extracts to inhibit microbial enzymes (amylase, protease, and lipase).

Materials and methods

Collection and extraction of plant samples

In the summer season of 2022, two plant species, *Rhazya stricta* and *Azadirachta indica*, were collected from the southern region of Jeddah Governorate, Kingdom of Saudi Arabia. The geographical coordinates of the collection sites were recorded as 20°.19´.18.6" N, 39°.20´.06.2" E for *R. stricta* and 21°.15´.42.8" N, 39°.10´.25.3" E for *A. indica*. The plant leaves of the species under investigation were subjected to extraction using the methodology described by Fazeli-Nasab *et al.* (2021). Initially, the leaves were cleaned with distilled water and then air-dried under ambient conditions, away from light. Subsequently, the dried leaves were finely ground into a powder. The extraction of *R. stricta* and *A. indica* leaves was performed using a solid-liquid extraction method. This involved using 2000 ml of methanol to extract 200 g of the air-dried leaf powder. The extraction process was repeated three

times, with each extraction lasting for 48 h. The resulting mixture was subjected to fluttering and drying using a rotary evaporator. Before testing, the extracts were diluted in a 1% solution of dimethyl sulphoxide (DMSO).

Analysis of active constituents using GC-MS

To determine the chemical composition of the leaf extracts, a Perkin Elmer model (Clarus 580/560S) mass spectrometer was employed. The column used was Elite-5MS (30 m, 0.25 mm ID, 0.25 µm df), and the oven temperature was controlled as follows: initially maintained at 35 °C, increased by 8 °C/min to 150 °C with a 3-min. hold, and then increased by 10 °C/min. to 280 °C. The inlet and transfer lines were maintained at 250 °C. Helium gas was used as the carrier gas at a flow rate of 1 ml/min. A solvent delay of 3 min. was applied, and diluted samples of 1 µl were automatically injected using Autosampler AS3000 in split mode. The mass spectrum was recorded at 70 eV ionization voltage over the range of m/z 40–650 in full scan mode. After column separation, the components were further analyzed using Flame Ionization Detection (FID). To determine the identities, molecular weights, and other properties of the compounds, their spectra were compared with established compounds in the NIST MS 2.0 structural library.

Evaluation of antibacterial activity

The methanolic leaf extracts of *R. stricta* and *A. indica* were tested for their antibacterial activity against four different bacterial strains: *Salmonella enteritidis* (ESBL700613), *Staphylococcus aureus* (ATCC25923), *Salmonella typhimurium* (ATCC14028), and *Escherichia coli* (NCTC9001) using the Well-diffusion technique. The bacterial strains were spread evenly over the surface of Muller-Hinton media using a sterilized glass rod. Six antibiotic discs, including Cefoxitin (FOX), Cephalothin (KF), Cotrimoxazole (TS), Gentamicin (GM), Augmentin (AUG), and Ampicillin (AP), were inserted using a sterile borer with a diameter of 5 mm. Saturated filter paper discs prepared from the leaf extracts were compared for their antibacterial activity with the antibiotic discs. The plates were then incubated at 37 °C for 24 h. After incubation, the clear zones around the discs were measured using a ruler to approximate the diameter of the inhibition zones in millimeters. The experiment was conducted in triplicate, and the average value of the three measurements (Clinical and Laboratory Standards Institute, 2018).

Monitoring bacterial enzymes activity

To assess amylase activity, the bacterial strains were cultured on a modified starch agar medium containing 0.25% starch. After incubation at 37 °C for 24 h, the presence of a clearing zone was detected by adding iodine as a detecting agent. The activity of protease was measured using skim milk plates consisting of 50.5 g of skim milk, 5 g of peptone, and 1 g of yeast extract per liter of distilled water. The presence of clear zones surrounding the colonies indicated protease activity. The lipase assay was conducted using an agar medium containing 2.5% agar, 2% Tween 20–80, and 0.01% Victoria Blue B. The cultured bacteria were incubated at a temperature of 30 °C within a circular well with a diameter of approximately 1 cm (Samad *et al.,* 1989).

Statistical analysis

The statistical software package SPSS (version 16) for Windows was utilized to calculate means, and standard errors, and perform analysis of variance (ANOVA) using a one-way design at a significance level of $P < 0.05$.

Results

GC-MS identification of leaf extracts active components

The results depicted in the Table 1 and Fig. 1 provided an overview of the identified active phytochemical compounds in the methanolic leaf extract of *Rhazya stricta* using gas chromatography-mass spectrometry (GC/MS) and the molecular weight approach. A total of 15 compounds were detected, namely spathulenol, octadecadiynoic acid, caryophyllene oxide, isoaromadendrene epoxide, heptatriacotanol,

dodecanoic acid, heptadecyn, hexadecenoic acid, pentadecanoic acid, octadecatrienoic acid, docosatetraenoic acid, octadecadienoyl chloride, aspidospermidine, and cyclic butylboronate. The abundance of these compounds in the leaf extract of *R. stricta* followed a specific order, with heptadecyn, spathulenol, and octadecatrienoic acid, 2,3-dihydroxypropyl ester, being the most prevalent. Aspidospermidine and octadecadienoyl chloride were also found to be present in substantial amounts. On the other hand, cyclic butylboronate, hexadecenoic acid, and heptatriacotanol were identified as the least abundant compounds.

Table 1. Phytochemical constituents in the leaf methanolic extract of *Rhazya stricta* as identified by GC-MS

Peak no.	RT (min.)	Compound name	Area (%)	Molecular formula	Molecular weight
1	16.03	Spathulenol	12.80	$C_{19}H_{30}O_2$	290
2	17.13	Octadecadiynoic acid, methyl ester	1.53	$C_{12}H_{26}OSi$	214
3	17.85	1h-3a,7-methanoazulene, Octahydro-3,8,8-trimethyl-6-methylene	2.34	$C_{15}H_{24}$	204
4	18.42	Caryophyllene oxide	4.59	$C_{15}H_{24}O$	220
5	24.29	Isoaromadendrene epoxide	0.89	$C_{15}H_{24}O$	220
6	24.36	Heptatriacotanol	0.74	$C_{37}H_{76}O$	536
7	25.60	Dodecanoic acid,	1.19	$C_{19}H_{34}O_6$	358
8	26.34	Heptadecyn	31.71	$C_{17}H_{32}0$	252
9	26.89	Hexadecenoic acid	0.72	$C_{17}H_{32}O_2$	268
10	27.32	Pentadecanoic acid	3.22	$C_{15}H_{30}O_2$	242
11	28.87	Octadecatrienoic Acid, 2,3-dihydroxypropyl Ester	10.3	$C_{21}H_{36}O_4$	352
12	30.12	Docosatetraenoic Acid, methyl ester	1.56	$C_{23}H_{38}O_2$	346
13	30.71	Octadecadienoyl chloride	9.11	$C_{18}H_{31}CIO$	298
14	31.80	Aspidospermidine	9.30	$C_{19}H_{24}N_2$	280
15	37.89	Cyclic butylboronate	0.38	$C_{25}H_{34}O_7$	446

RT = Retention time

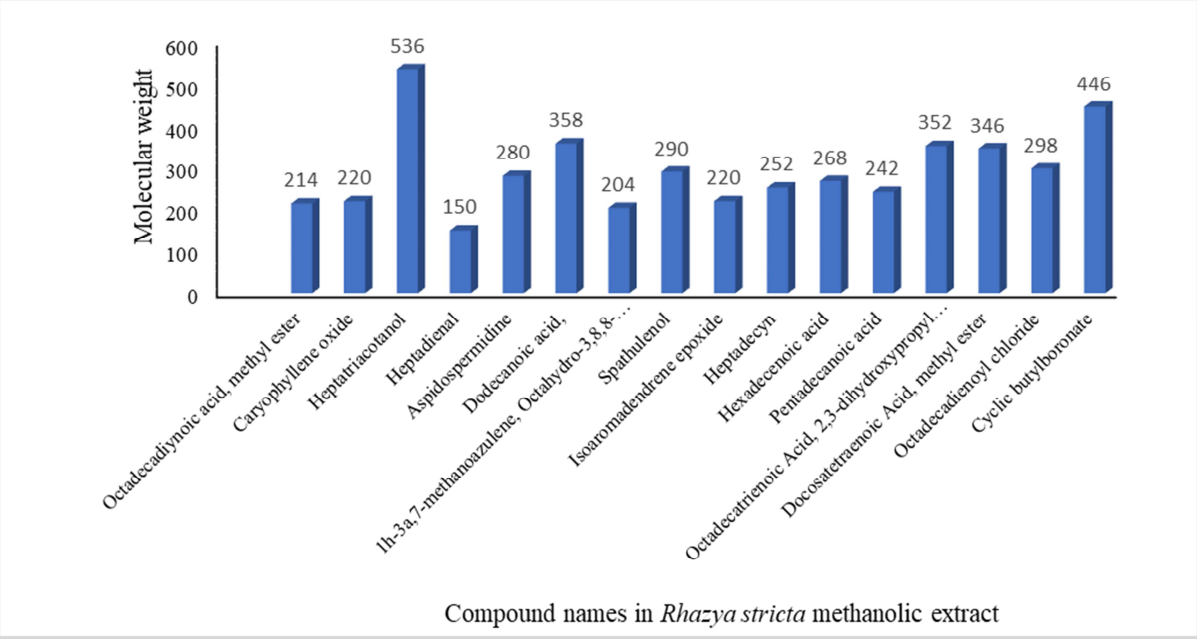

Fig. 1. Molecular weight of compounds identified in *Rhazya stricta* leaf methanolic extract

In a similar vein, Table 2 and Fig. 2 provide an insight into the active components present in the methanolic extract of *Azadirachta indica*, as detected using the GC/MS and the molecular weight technique. The data revealed the presence of 32 active phytochemicals in the leaf extract of *A. indica*. These compounds included heptadienal, carbonitrile, tetradecadiynoate, heptadecynyloxy,

octadecadiynoic acid, cyclopropaneoctanoic acid, picrotoxin, octadecatrienoic acid, pentacosadiynoic acid, caryophyllene oxide, epiglobulol, octadecanal, retinal, eicosapentaenoic acid, octadecenoic acid, hydroxyand rostane, heptatriacotanol, aspidospermidine, oxiraneundecanoic acid, isochiapin, hexadecanoic acid, isopropyl palmitate, cholestan, eicosenoic acid, cyclopropaneoctanoic acid, octanoic acid, docosatetraenoic acid, eicosatetraenoic acid, heptadecen, and benzopyran. Among these compounds, isopropyl palmitate, cyclopropaneoctanoic acid, cholestan, octadecadiynoic acid methyl ester, and hexadecanoic acid were found to be the most abundant. Conversely, some phytochemicals such as hydroxyand rostane, heptadienal, 2-ethylidene-6-methyl, octadecanal, eicosatetraenoic acid methyl ester, eicosenoic acid, heptadecen, and cyclopropaneoctanoic acid were detected in trace quantities.

Table 2. Phytochemical constituents in the leaf methanolic extract of *Azadirachta indica* as identified by GC-MS

Peak no.	RT	Compound name	Area (%)	Molecular formula	Molecular weight
1	15.76	Heptadienal, 2-ethylidene-6-methyl	0.41	$C_{10}H_{14}O$	150
2	16.06	Heptadienal	1.16	$C_{10}H_{14}O$	150
3	16.33	Carbonitrile	1.28	$C_{20}H_{27}NO_2$	313
4	16.69	Tetradecadiynoate	1.50	$C_{15}H_{22}O_2$	234
5	17.53	Heptadecynyloxy	1.91	$C_{22}H_{40}O_2$	336
6	18.77	Octadecadiynoic acid, methyl ester	6.46	$C_{19}H_{30}O_2$	290
7	19.30	Cyclopropaneoctanoic acid	0.62	$C_{22}H_{38}O_2$	334
8	19.39	Picrotoxin	0.66	$C_{15}H_{16}O_6$	292
9	19.69	Octadecatrienoic acid	1.43	$C_{27}H_{52}O_4Si_2$	496
10	19.95	Pentacosadiynoic acid	0.71	$C_{25}H_{42}O_2$	374
11	20.03	Caryophyllene oxide	0.71	$C_{15}H_{24}O$	220
12	20.23	Epiglobulol	1.75	$C_{15}H_{26}O$	222
13	20.68	Octadecanal	0.46	$C_{18}H_{36}O$	270
14	20.97	Retinal	2.04	$C_{20}H_{28}O$	284
15	21.10	Eicosapentaenoic acid	1.09	$C_{20}H_{30}O_2$	302
16	23.46	Octadecenoic acid	2.14	$C_{18}H_{34}O_2$	282
17	23.90	Hydroxyand rostane	0.23	$C_{19}H_{24}O_3$	300
18	23.96	Heptatriacotanol	0.43	$C_{37}H_{76}O$	536
19	24.31	Aspidospermidine	1.81	$C_{21}H_{26}N_2O_2$	338
20	25.29	Oxiraneundecanoic acid	3.75	$C_{19}H_{36}O_3$	312
21	25.64	Isochiapin	0.81	$C_{19}H_{22}O_6$	346
22	26.56	Hexadecanoic acid	4.65	$C_{18}H_{36}O_2$	284
23	27.08	Isopropyl palmitate	14.32	$C_{19}H_{38}O_2$	298
24	28.56	Cholestan	9.32	$C_{28}H_{48}O$	400
25	28.98	Eicosenoic acid	0.59	$C_{20}H_{38}O_2$	310
26	29.75	Cyclopropaneoctanoic acid	11.73	$C_{22}H_{38}O_2$	334
27	30.74	Octanoic acid	0.14	$C_{21}H_{38}O_2$	322
28	30.90	Cyclopropaneoctanoic acid	0.70	$C_{22}H_{38}O_2$	334
29	32.50	Docosatetraenoic acid, methyl ester	0.19	$C_{23}H_{38}O_2$	346
30	32.60	Eicosatetraenoic acid, methyl ester	0.50	$C_{21}H_{34}O_2$	318
31	35.42	Heptadecen	0.64	$C_{18}H_{30}O_2$	278
32	36.31	Benzopyran	1.53	$C_{27}H_{30}O_{16}$	610

RT = Retention time

Comparative assessment of antibacterial properties

The inhibitory potential of the methanolic extracts from *A. indica* and *R. stricta*, in comparison with commercially available antibiotics, was evaluated through *in vitro* experiments. Table 3 & 4 and Fig. 3 depict the results of these investigations. The findings indicated that both plant species' methanolic extracts exhibited remarkable effectiveness in inhibiting the growth of all tested microorganisms. In the case of *R. stricta*, the order of efficiency against the bacterial strains was as follows: *S. typimurium* > *E. coli* > *S. enteridis* > *S. aureus*, with corresponding inhibition zones measuring 4.3, 4.2, 4.0, and 3.0 cm, respectively. Moreover, the methanolic extract of *A. indica* demonstrated greater efficacy against the bacterial strains compared to that of *R. stricta*.

Among the tested strains, *S. enteridis* and *S. typimurium* displayed the highest sensitivity (with inhibition zones of 4.8, 4.6, and 3.5 cm, respectively), followed by *E. coli* and *S. aureus*. In contrast to the inhibition zones generated by the examined antibiotics, the extracts from both plant species exhibited significantly enhanced and prominent antibacterial efficacy against *S. enteritidis, S. typhimurium,* and *E. coli*. This was evident from the observation that the diameter of inhibition zones formed by these extracts surpassed those generated by all the antibiotics under investigation. The ANOVA statistical analysis conducted on this particular section confirmed the evident impact on nearly all the tested microorganisms, with only a slight variation observed in the case of *E. coli*.

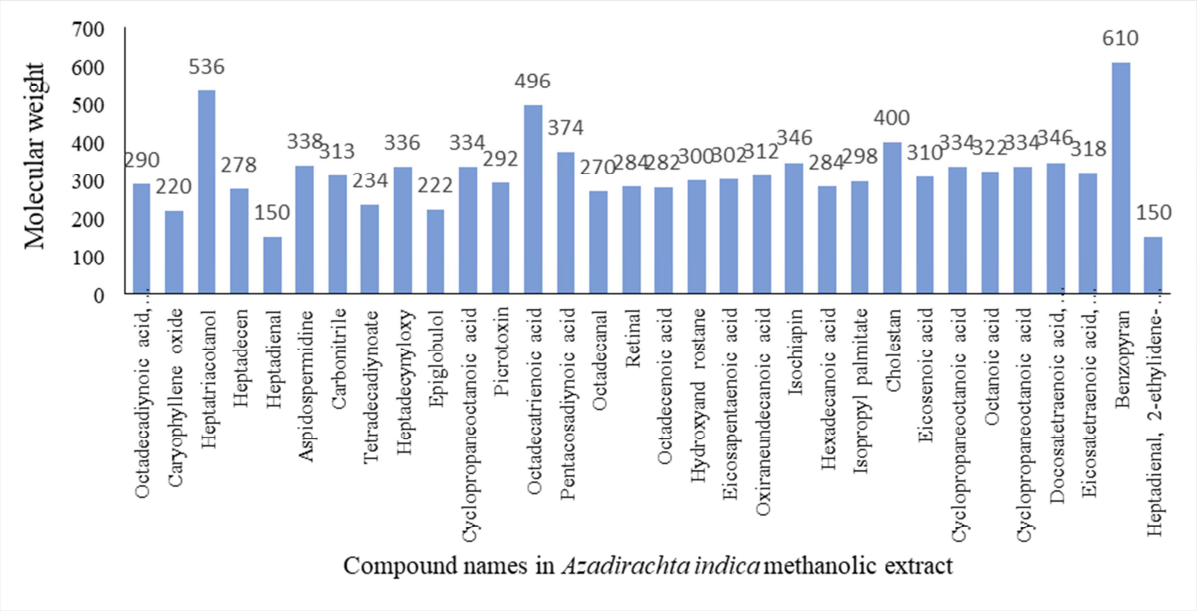

Fig. 2. Molecular weight of compounds identified in *Azadirachta indica* leaf methanolic extract

Table 3. Antimicrobial activity of *Rhazya stricta* and *Azadirachta indica* leaves extracts (cm)

Microorganisms	*Rhazya stricta*		*Azadirachta indica*		Antibiotics					
	Methanolic extract	Water extract	Methanolic extract	Water extract	FOX	KF	GM	TS	AUG	AP
S. enteritidis	4.0±0.15^{a}	1.0±0.10^{c}	4.8±0.20^{a}	2.0±0.10^{a}	3.0	2.5	2.5	2.6	2.5	2.0
Staph. aureus	3.0±0.20^{b}	1.5±0.10^{a}	3.5±0.12^{c}	1.0±0.10^{d}	3.0	00	3.5	3.5	3.5	4.5
S. typhimurium	4.3±0.20^{a}	0.8±0.06^{d}	4.8±0.12^{a}	1.3±0.06^{c}	3.4	2.5	2.0	2.5	2.5	2.0
E. coli	4.2±0.10^{a}	1.3±0.10^{b}	4.6±0.06^{b}	1.5±0.12^{b}	2.5	1.5	2.5	2.5	2.5	1.5

Different letters within the same column represent significant differences at $P < 0.05$.

Table 4. One-way ANOVA statistical analysis of the antimicrobial activity of *R. stricta* and *A. indica* leaf extracts

Micro-organisms	Comparison	*Rhazya stricta*		*Azadirachta indica*		Antibiotics					
		Meth. extract	Water extract	Meth. extract	Water extract	FOX	KF	GM	TS	AUG	AP
Salmonella enteritidis	Mean difference	3.00	0.85	3.85	2.85	1.00	1.50	1.50	1.40	1.50	2.00
	Standard error	0.09	0.09	0.13	0.08	0.08	0.08	0.08	0.08	0.08	0.08
	Standard deviation	0.29	0.29	0.39	0.26	0.26	0.26	0.25	0.26	0.26	0.26
	95% confidence from	2.57	1.28	4.28	2.42	1.43	1.07	1.07	0.97	1.07	1.57
	to	3.43	0.42	3.42	3.28	0.57	1.93	1.93	1.83	1.93	2.43
	Q test	35.9	10.2	46.1	43.1	11.9	17.9	17.9	16.8	17.9	23.9
	Normality test KS	0.1000	0.1000	0.25	0.1000	0.1000	0.24	0.1000	0.1000	0.1000	0.1000
	Normality test *P value*	*P*<0.001 ***	*P*<0.001 ***	*P*<0.001 *	*P*<0.001 ***	*P*<0.001 ***	*P*<0.001 *	*P*<0.001 *	*P*<0.001 ***	*P*<0.001 ***	*P*<0.001 ***
Staph. aureus	Mean difference	1.50	2.00	2.00	0.50	0.00	3.00	0.50	0.50	0.50	1.50
	Standard error	0.08	0.08	0.08	0.08	0.00	0.08	0.08	0.08	0.08	0.08

	Standard deviation	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
	95% confidence from	1.07	1.57	2.43	0.07	0.00	2.57	0.07	0.07	0.07	1.93
	95% confidence to	1.93	2.43	1.57	0.93	0.00	3.43	0.93	0.93	0.93	1.07
	Q test	17.9	23.9	23.9	5.99	0.00	35.9	5.99	5.99	5.99	17.9
	Normality test KS	0.1000	0.1000	0.1000	0.1000	0.000	0.1000	0.1000	0.1000	0.1000	0.1000
	Normality test *P* value	*P*<0.001**	*P*<0.001***	*P*<0.001*	*P*<0.001**	*P*<0.00 ss	*P*<0.001**	*P*<0.001*	*P*<0.001***	*P*<0.001***	*P*<0.001***
Salmonella typhimurium	Mean difference	4.3	0.80	4.80	1.30	3.40	2.50	2.00	2.50	2.50	2.00
	Standard error	0.06	0.08	0.08	0.60	0.08	0.08	0.08	0.08	0.08	0.08
	Standard deviation	0.18	0.26	0.26	0.18	0.26	0.26	0.26	0.26	0.26	0.26
	95% confidence from	3.09	0.91	0.49	1.39	1,89	1.89	1.89	2.70	2.70	1.89
	95% confidence to	3.91	0.93	1.31	2.21	2.70	2.70	2.70	28.9	28.9	2.70
	Q test	43.9	6.28	37.7	11.3	22.6	28.9	22.6	22.6	22.6	28.9
	Normality test KS	0.1080	0.1000	0.1000	0.1080	0.000	0.1000	0.1000	0.1000	0.1000	0.1000
	Normality test *P* value	*P*<0.001**	*P*<0.001**	*P*<0.001*	*P*<0.001***	*P*<0.001***	*P*<0.001**	*P*<0.001*	*P*<0.001***	*P*<0.001***	*P*<0.001***
E. coli	Mean difference	4.20	1.30	4.60	1.50	2.50	1.50	2.50	2.50	2.50	1.50
	Standard error	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
	Standard deviation	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
	95% confidence from	2.49	0.81	2.29	1.29	1.29	2.29	1.29	1.29	1.29	2.29
	95% confidence to	3.31	0.00	3.11	2.11	2.11	3.11	2.11	2.11	2.11	3.11
	Q test	36.4	5.03	33.9	21.4	33.9	35.9	21.4	21.4	21.4	33.9
	Normality test KS	0.1000	0.1000	0.1000	0.1000	0.000	0.1000	0.1000	0.1000	0.1000	0.1000
	Normality test *P* value	*P*<0.001**	*P*<0.05n s	*P*<0.001*	*P*<0.001***	*P*<0.001***	*P*<0.001**	*P*<0.001*	*P*<0.001***	*P*<0.001***	*P*<0.001***

Meth. extract =Methanolic extract

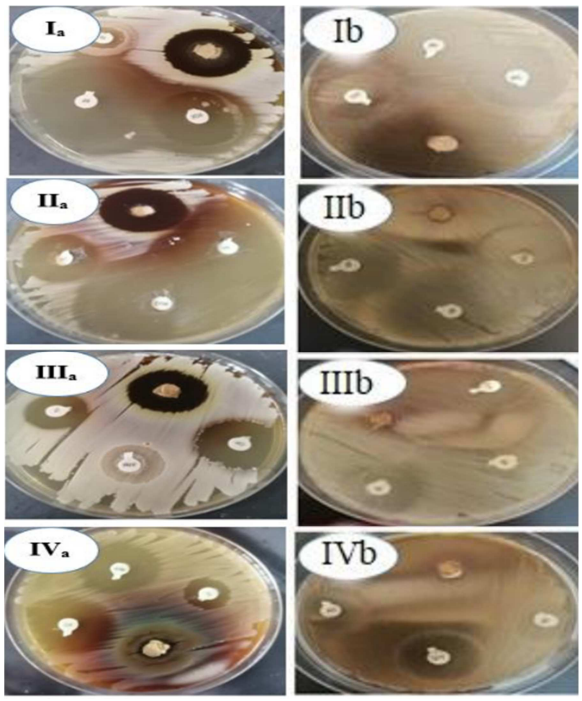

Fig. 3. Antimicrobial activity of the methanolic leaf extracts of *R. stricta* and *A. indica* against the investigated bacterial strains

(I) *S. enteritidis*; (Ia) methanolic extract of *R. stricta*, (Ib) methanolic extract of *A. indica*.

(II) *S. aureus*; (IIa) methanolic extract of *R. stricta*, (IIb) methanolic extract of *A. indica*.

(III) *S. typhimurium*; (IIIa) methanolic extract of *R. stricta*, (IIIb) methanolic extract of *A. indica*.

(IV) *E. coli*; (IVa) methanolic extract of *R. stricta*, (IVb) methanolic extract of *A. indica*.

Impact of leaf extracts on microbial enzymes

Fig. 4 illustrates the inhibitory effects of the methanolic extracts derived from *A. indica* and *R. stricta* on three microbial enzymes: amylase, protease, and lipase.

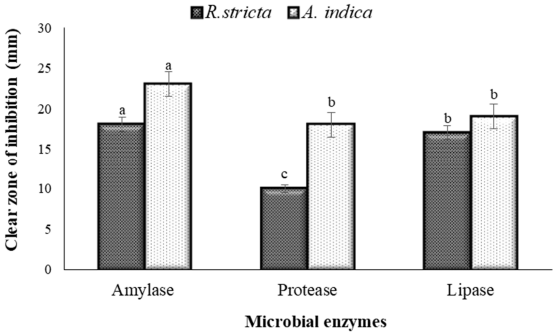

Fig. 4. Comparison between the methanolic extracts of *R. stricta* and *A. indica* against the investigated bacterial enzymes. Different letters within the same extract represent significant differences at $P < 0.05$

The study's findings revealed the inhibitory effects of these extracts on all examined bacterial enzymes. Notably, the inhibitory impact was most pronounced on amylase, followed by lipase and protease. In particular, the extract of *R. stricta* displayed inhibitory effects of 18, 17, and 10 mm on amylase,

lipase, and protease, respectively. Similarly, *A. indica* exhibited inhibitory effects on these enzymes, with inhibition zones measuring 23, 19, and 18 mm, respectively. Overall, the inhibitory impact of *A. indica* was found to be more pronounced than that of *R. stricta* on all tested microbial enzymes.

Discussion

Our study's main objective was to look into the antibacterial properties of *R. stricta* and *A. indica* as possible alternatives to commercially available antibiotics in order to avoid the side effects of these antibiotics and stop foodborne pathogens from becoming more resistant to them. The study evaluated the effectiveness of *R. stricta* and *A. indica* methanolic extracts against the most prevalent foodborne bacteria that are virtually resistant to conventional antibiotics. The obtained findings demonstrated that *A. indica* had a greater inhibitory impact on the tested Gram-negative bacteria compared to the Gram-positive species, thereby establishing it as the most efficacious extract against all the microorganisms examined. As a result, it was observed that *R. stricta* and *A. indica* exhibited comparable levels of inhibition against the microbial enzymes under investigation. However, it is noteworthy that *A. indica* displayed the highest efficacy in inhibiting all tested microbial enzymes. Both plant extracts exhibited a higher degree of microbial amylase inhibition, followed by lipase and protease enzymes, respectively.

The study conducted by Sufiyanu *et al.* (2021) established that the methanol extract of *A. indica* leaves has significant antimicrobial activity against *S. aureus*. Moreover, the study conducted by Hemdan *et al.* (2023) revealed that extracts derived from the leaves of *A. indica* have significant antibacterial properties against *S. aureus*. In a study conducted by Sharma *et al.* (2024), they observed the antibacterial properties of the extract of *A. indica* leaves. The extract exhibited significant efficacy against both Gram-negative and Gram-positive bacteria, with a particular emphasis on its effectiveness against gastrointestinal infections. In their study, Selvamohan *et al.* (2012) documented the significant impact of *A. indica* leaf extract, particularly its efficacy against Salmonella spp., *E. coli*, and *S. aureus*. The antibacterial activity of *A. indica* was attributed to the presence of quinone compounds that can bind to bacterial cell polypeptides and interact with bacterial enzymes, thereby impeding the proliferation of bacterial cells (Álvarez-Martínez *et al.,* 2024). Additionally, Mudenda *et al.* (2023) reported the presence of flavonoids, saponins, and tannins in *A. indica* extract, which exhibit significant inhibitory properties against the enzymes of *S. aureus*, *E. coli*, and other pathogens. All in all, the antimicrobial characteristics of *A. indica* could be attributed to its phytochemical components.

The findings of our investigation align with those of Bukhari *et al.* (2017), as they observed that *R. stricta* exhibited antibacterial properties by interacting with proteins and carbohydrates present in the bacterial cell wall, therefore rendering bacterial enzymes inactive. The efficiency of *R. stricta* against several bacterial strains, including *S. aureus*, *E. coli*, *Bacillus* spp., *K. pneumonia*, *Proteus* spp., *P. aeruginosa*, *Salmonella* spp., and MRSA, was documented by Bukhari *et al.* (2017), Hassan *et al.* (2023), and Haq *et al.* (2024). The study conducted by Ahmed *et al.* (2022) revealed a diminished impact on Gram-negative bacteria. In contrast, Al Akeel *et al.* (2017) asserted that the ethanolic extract of *R. stricta* has a high potency, making it a potentially effective broad-spectrum antibacterial agent. The efficacy of *R. stricta* can be attributed to the presence of many active compounds, including flavonoids, alkaloids, cardenolides, saponins, and other bioactive components (Dillard and German, 2000; Alzamel, 2022).

There are several promising avenues for future research aimed at developing practical therapeutic regimens utilizing *R. stricta* and *A. indica* for human use. Further investigation will be necessary to elucidate the mechanisms by which *A. indica* and *R. stricta*, as well as their associated phytochemicals, exert their effects. Based on the findings presented in this study, it is clear that the use of distinct

phytochemicals derived from *R. stricta* and *A. indica* holds significant potential as antimicrobial agents. These phytochemicals can be employed either independently or in combination with the existing antimicrobial agents. Furthermore, further pre-clinical and clinical investigations are necessary to evaluate the toxicity and optimal in vivo dosing of specific phytochemicals in comparison to the original plant extracts. These areas of research demonstrate considerable promise for future exploration.

It is important to emphasize the need to thoroughly evaluate the types of extracts, including both the specific plant component and the solvent, that have been previously examined for their efficacy against various species. This meticulous examination is crucial to maximizing the potential benefits derived from these fields of scientific investigation. To facilitate the identification of trends across several research studies and enable meaningful comparisons, it is necessary to consider a certain degree of uniformity. The identification of antibacterial capabilities in phytochemicals would provide a more straightforward analysis when these compounds are more precisely characterized. The extracts of *R. stricta* and *A. indica* are potential sources of antimicrobial agents that might be utilized in addressing the challenges posed by antimicrobial resistance and developing risks to human health. Moreover, the existing research on the species *R. stricta* and *A. indica* can serve as a valuable reference to stimulate further exploration into the potential of other historically employed natural products in the context of contemporary medicinal applications.

In the present investigation, the methanolic extracts obtained from *A. indica* and *R. stricta* were examined for their impact on three microbial enzymes: amylase, protease, and lipase. Some previous studies have reported the inhibitory effect of *A. indica* extract on microbial enzymes' activity (Wolinsky *et al.,* 1996; Gopal *et al.,* 2007; Bodiba *et al.,* 2018). The inhibitory mechanism of *A. indica* was attributed to its diverse phytochemical composition, including azadirachtin, nimbin, and salanin (Gopal *et al.,* 2007). These compounds have been found to hinder bacterial aggregation, growth, adhesion, and the production of insoluble glucan, potentially affecting the formation of plaque in vitro (Wolinsky *et al.,* 1996). Furthermore, the extract of neem leaves showed potential antibacterial activity against both beta-lactamase-producing and nonproducing Gram-negative bacilli (Faujdar *et al.,* 2020).

Recent studies have shown that *R. stricta* extract possesses potent antibacterial activity, inhibiting the growth of a wide range of bacterial strains. One of the mechanisms through which *R. stricta* extract exerts its antibacterial effect is by inhibiting the activity of bacterial enzymes. Bacterial enzymes play crucial roles in various metabolic processes and are essential for the survival and growth of bacteria. By targeting these enzymes, *R. stricta* extract disrupts the normal functioning of bacteria, thereby inhibiting their growth (Hassan *et al.,* 2023). This mechanism of action is particularly effective because it targets multiple enzymes, making it difficult for bacteria to develop resistance. Some studies have investigated the specific enzymes inhibited by *R. stricta* extract. Alqarawi *et al.* (2018) reported that *R. stricta* extract inhibited the activity of enzymes involved in cell wall synthesis, such as penicillin-binding proteins (PBPs). This disruption of cell wall synthesis weakens the structural integrity of bacterial cells, making them more susceptible to destruction by the immune system or other antimicrobial agents.

Moreover, *R. stricta* extract has also been found to inhibit enzymes involved in nucleic acid synthesis, protein synthesis, and energy metabolism (Sultana and Khalid 2010). By interfering with these essential processes, the extract effectively halts bacterial growth and proliferation.

The inhibitory effect of *A. indica* and *R. stricta* extracts on bacterial enzymes highlights their potential as natural antibacterial agents. However, further research is needed to elucidate the specific compounds responsible for their activity and to determine their precise mechanisms of action.

Understanding these mechanisms will not only contribute to the development of new antibacterial drugs but also shed light on the potential applications of natural plant extracts in combating bacterial infections.

Conclusion

In conclusion, our investigation has demonstrated the significant antibacterial activity of methanolic leaf extracts derived from *R. stricta* and *A. indica* against the tested bacterial strains, specifically *Salmonella typhimurium* and *Salmonella typhi* strains. The observed medicinal efficacy of these plant extracts can be attributed to the presence of biologically active secondary metabolites within their leaf extracts, such as spathulenol, octadecatrienoic acid, aspidospermidine, and octadecadienoyl chloride. Thus, the leaf extracts of *R. stricta* and *A. indica* possess the ability to interact with various components of bacterial cells, thereby impeding their growth. This inhibitory effect is achieved through the suppression of the biosynthesis of key microbial enzymes like amylase, protease, and lipase. These findings suggest that these plant extracts have the potential to serve as antimicrobial agents against bacterial pathogens, with particular emphasis on *A. indica*. However, further research is warranted to elucidate the specific mechanisms of action and explore their therapeutic applications in combating drug-resistant bacterial infections.

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