Pol. J. Environ. Stud. Vol. XX, No. X (XXXX), 1-8
DOI: 10.15244/pjoes/190564 ONLINE PUBLICATION DATE:

Original Research

Plant Based Antimicrobials Against Multi Drug Resistant Bacterial Strains (MDRS)

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Received: 13 November 2023
Accepted: 27 June 2024

Abstract

Plants are vital for life due to their various constituents, comprised of various parts like roots, stems, and leaves. Herbal medicines have been known to man for centuries. The study was designed to check and compare the antibacterial activity of onion (*Allium cepa* L.), neem (*Azadirachta indica* A. Juss.), and bitter gourd (*Momordica charantia* L.) methanolic extracts against four bacterial strains, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. All bacterial strains were cultured on nutritional agar plates. Three parameters were used in this research: concentrations, pH, and temperature. The five concentrations, 50 mg/mL, 70 mg/mL, 90 mg/mL, 110 mg/mL, and 130mg/mL were applied to all bacterial strains. At 110mg/mL, the methanolic extract of neem and bitter gourd applied to *E.coli* and *S. aureus* showed the largest zone of inhibition of 1.8 mm. The minimum inhibitory concentration (MIC) for *Bacillus subtilis* was 50 mg/mL when onion methanolic extract was applied. Onion and neem extract was found effective at 100 ^{0}C against *E.coli* and *B. subtilis,* while bitter gourd controls the growth of *E.coli* at 60 ^{0}C. The results of variable pH revealed that onion and bitter gourd were effective at pH 7 against *B. subtilis,* while neem was effective against *S. aureus* at pH 2.

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The results of the present study suggest that all onion, neem, and bitter gourd extracts have compounds containing antibacterial properties that can potentially be useful to control food-borne pathogens. By comparing the extracts of selected plants, neem was found to be more effective than onion and bitter gourd.

Keywords: concentrations, E. coli, foodborne, plant, spices

Introduction

Green plants are not only the basic source of nutrition for almost all living things, but the secondary metabolites produced by the plants serve as the basic source of medication as well [1]. Medicinal plants, as a whole or their parts, like leaves, roots, seeds, fruit, and bark, contain phytochemicals, which are used for medicinal purposes and therefore are precursors for the production of drugs [2].

A. indica (Neem tree) belongs to the Meliaceae family and is widely distributed throughout South Asia, including Bangladesh, Pakistan, and India. Nepal, and Sri Lanka, exhibit extremely beneficial medicinal properties [3, 4]. Neem grows to a maximum height of 25 meters, with a straight or semi-straight stem, and the branches are spread to make a broad crown [5]. Neem is used in Unani, Ayurveda, as well as in homeopathic medicine. Extracts of various parts of *A. indica*, such as bark, leaf, seed, and fruit, have been tested and found to possess antimicrobial potential against pathogenic microorganisms [6, 7]. The plant has become a cynosure of Unani medicine employed in the treatment of diabetic mellitus, viral infections, and fungal diseases like eczema/acne, ringworm, and inflammation [7-10]. Aqueous extract of Neem bark was reported for its antiulcer and antisecretory potential by [11, 12] showing that acetone-water extract is useful for reducing the viral load and the restoration of $CD4^+$ cells.

A. cepa (Onion), cultivated throughout the world, is a culinary herb with an underground fleshy bulb. The plant belongs to the family Liliaceae which is usually cultivated as a spice, but it has a long history of medicinal uses [1]. Various Allium species have been used as medicinal plants in different parts of the world due to their bioactive constituents that cure many ailments and therefore prove beneficial to human health [13]. The onions contain an excellent proportion of distinct and highly valuable classes of phytochemicals, including flavonoids, organosulfur compounds, minerals, and polysaccharides. A diverse array of these phytochemicals contained by this universal crop imparts some very useful biological properties, like antioxidant, anti-inflammatory, and antibacterial activity [14-16]. Tsuboki et al. [17] reported a direct reduction of cell proliferation in ovarian cancer by onionin [17]. Neuroprotective effects of the ethanolic extract of onion were studied by [18, 19] and reported the benefits of onion peel extract for weight loss applications [18, 19].

The bitter gourd, scientifically known as *Mordica charantia L.*, has 22 loculi and is a member of the Cucurbitaceae family. The bitter gourd is mostly thought to have originated in India [20]. It was likely domesticated in southern China or eastern India in eastern Asia. Tender fruits are used as vegetables and several processed products. Most bitter gourd plants are monoecious, meaning they produce staminate and pistillate flowers. However, several reports from Japan, India, and China indicate that some varieties are gynecious, indicating they solely produce pistillate flowers. Fruits are considered to be the most nutritious vegetable among cucurbits due to their high content of essential nutrients, including carbohydrates, proteins, minerals, and vitamins, with ascorbic acid and iron. In addition to these nutrients, bitter gourd also contains compounds that give it its bitter flavor, such as phenols, terpenes, saponins, and glucosinolates. Fruits offer potential benefits in managing blood disorders, rheumatism, diabetes, and asthma [21].

Due to the unparalleled variety of chemicals found in natural products, whether in the form of pure chemicals or standardized plant extracts, herbal treatment is extremely important and offers countless opportunities for novel therapeutic approaches. Finding novel antimicrobial agents with distinct chemical structures and modes of action is a continuous and critical need for emerging and reemerging infectious diseases [22]. Because of this, scientist’s attention is turning more and more to conventional medicine for inspiration when developing novel antimicrobial drug formulations [23]. Many medicinal plants have been evaluated for their antimicrobial activity due to the failure of chemotherapeutics and the emergence of antibiotic resistance in pathogenic microbial infectious agents. Therefore, it is anticipated that phytochemicals with sufficient antibacterial efficacy will be employed in the management of bacterial infections [24]. Different plant parts have been used by humans to treat and prevent a wide range of illnesses [5].

Due to its antiviral properties, neem is also used to treat viruses, chikungunya, measles, and coxsackie [25]. Additionally, it is used to treat a variety of illnesses, including ringworm, gas gangrene, eczema, acne, and inflammation. The extracts from its leaves are also used to treat diabetes mellitus. Neem also possesses pharmacological qualities such as anti-diabetic, anti-ulcer, anti-carcinogenic, antioxidant, and anti-malarial. The presence of numerous bioactive compounds in its different parts is thought to be responsible for the biological activity of plants. Neem purifies blood by neutralizing free radicals and returning them to their normal state within the body [26]. Neem's antioxidant activity has a positive impact on the central nervous system and is also used to treat antifertility issues. Neem is also used in the treatment of cancer and AIDS because it boosts immune system function. Studies have shown that neem lowers blood sugar levels and is effective in reducing insulin usage in diabetic patients by 30% to 50% [27].

Allium cepa has a vast array of phytocompounds that have been observed, including flavonoids, phenolic acids, a thyocyanin, and an organosulfur component. Athletes used onions to purify their blood during the Greek and Roman eras, and onion extract was used to build muscle in Rome. Both wound healing and the treatment of pneumonia were advised by the ancient Greek physicians using onions. In India in the sixth century, onions were considered both a vital vegetable and spice. Additionally resistant to cardiovascular disease, onion extracts exhibit thrombolytic, antioxidant, and hypocholesterolemia-based anticancer properties [28].

The phytochemical prospects of the dried and fresh leaf extracts of bitter gourd revealed the presence of several secondary metabolite classes with antimicrobial activity, such as tannins, alkaloids, and flavonoids. Both fresh and dried leaves showed notable antimicrobial activity against every strain of bacteria tested, particularly *E.coli. B. cereus* and *E. coli* were both susceptible to the effects of ethyl acetate fractions. Additionally, the modulatory activity was significant [29].

Tropical wines like bitter gourd are primarily grown in Southeast Asia, China, and India. The edible fruit portion of the plant is the primary reason it is grown. The bitter taste of bitter gourd makes it unpopular with most people. Still, the fruit provides a number of important nutrients. More than 60 phytomedicines, including those effective against diabetes and cancer, are present in the entire plant. The use of the bioactive substances that have been separated from bitter gourd in functional meals and drinks is currently expanding [21]. Therefore, the present study was conducted to check the efficacy of methanolic extracts of neem leaves and onion fruit against some very notorious food poisoning bacteria.

Materials and Methods

Bacterial Strains

Four bacterial strains selected for this study include one Gram-negative bacteria, *E. coli,* and three Gram-positive strains, *S. aureus*, *B. cereus,* and *B. subtilis*. All these four bacterial strains are facultative anaerobic organisms, which were identified and collected in the following manner: *B. cereus* was obtained from a rice field, *B. subtilis* was separated from the soil, *S. aureus* was separated from fresh milk, and *E. coli* was isolated from fecal-contaminated water, obtained from different areas of Sargodha city.

A Sampling of Plant Material

Onion (*A. cepa*) was purchased from the local market of Sargodha; bitter gourd was purchased from Khushab, District Sargodha; and neem (*A. indica*) leaves were collected from the Neem trees in Sargodha city. The fruit part of the onion, bitter gourd, and leaves of neem were used in this study. The fruit part of the onion was cut into small pieces and then shade dried in a room at room temperature (37°C). bitter gourd fruit was washed and sun dried. The Neem leaves were collected and thoroughly washed with water, followed by screening of healthy and fresh leaves, which were dried in the room at a room temperature of 37°C. The dried leaves of Neem and the fruit part of the onion and bitter gourd were ground to a fine powder using a blender, followed by preparing the methanolic extract following the protocol from [30].

Preparation of Methanolic Extracts

Onion, bitter gourd, and neem were extracted from methanol using the Soxhlet extraction method [31]. In the extraction chamber, which was suspended above the flask containing the methanol and below a condenser, the ground plant material was put. The solvent in the flask evaporated as it was heated, and then it flowed to the condenser and changed into a liquid that dripped into the extraction chamber containing the plant material. The extraction chamber was made to have an overflowing solvent that trickled back down into the boiling flask when the solvent level around the sample surpassed a certain level. The flask contains the methanolic extract of the plant. For the separation of methanol from plant extract using a rotary evaporator at 20°C. The extract contains a small amount of methanol. The remaining extract was dried at room temperature and took about 15 days to dry completely. Extracts were stored in the freezer at a temperature of 4°C. The same procedure was repeated for all the selected plants.

Maintenance of Bacterial Culture and Inoculum Preparation

Pure cultures of the bacterial strains were grown, maintained, and refreshed on nutrient agar media and plates. The cultures were streaked on sterile nutrient agar plates and incubated at 37°C for 24 h. In order to prevent contamination, the bacterial cultures were refreshed every three to four days. Each of the pure bacterial cultures was grown overnight at 37°C in nutrient broth to create the inoculum.

Inhibition Zone Assay

The methanolic extracts of bitter gourd, neem, and onion were used to check their antibacterial potential against the selected bacterial strains by well diffusion assay following the method of [32] with minor modifications. Briefly, bacterial strains were cultured using the serial dilution method. 1 mL of each strain was poured on its respective plate, and then, using the spreader, colonies of bacteria were loaded smoothly on their respective plates. Then extract was diluted by using deionized water to obtain the required concentrations. Then the wells of about 6 mm diameter were bored using cork borers on the plates, and the diluted extract was poured into these wells in the order of increasing concentrations. Deionized water was loaded on the plates as a negative control, while Amoxil (500 mg) was used as a positive control to observe the antibacterial potential of selected plants. The culture medium was incubated for one day at 37°C, followed by measuring the inhibition zones using a vernier caliper [33].

Minimum Inhibitory Concentration Assay

Five different concentrations, 50 mg/mL, 70 mg/mL, 90 mg/mL, 110 mg/mL, and 130 mg/mL of methanolic extracts of the two plants under investigation were applied against the selected bacterial strains. The minimum inhibitory concentration (MIC) of the two methanolic extracts was determined following the protocol of [32] and proceeded as given in the above section. This inhibition zone assay experiment was performed in triplicate and calculated the MIC [24, 33].

Effect of Temperature on Antibacterial Activity of Plant Extract

The effect of temperature on the antibacterial potential of methanolic extracts of neem leaves and onion bulbs was investigated at five different temperatures. Two different concentrations, 50 mg/mL and 130 mg/mL, of each of the two extracts were placed for 30 min in water baths, already set at five different temperatures: 20°C, 40°C, 60°C, 80°C, and 100°C.

Effect of pH on Antibacterial Activity of Plant Extract

The effect of pH on methanolic extracts under investigation was proceeded by testing the two concentrations, 50 mg/mL and 130 mg/mL, of each extract at five different pH values (2, 5, 7, 9, and 13). The given pH values of the solutions under investigation were adjusted by adding sulfuric acid or sodium hydroxide.

Statistical Analysis

The data are expressed as the mean ± SD, and significant differences of triplicate measurements between mean values were determined by one way ANOVA followed by Tukey’s test with one way analysis of variance.

Results and Discussion

The antibacterial activity of methanolic extracts of onion bulb, bitter gourd, and neem leaves was checked against selected Gram-positive and Gram-negative bacterial strains. The zones of inhibition shown by extracts under investigation at different concentration ranges are shown in Table 1. The maximum zone of inhibition of 1.8 mm was observed by neem and bitter gourd methanolic extracts applied at a concentration of 110 mg/mL against *S. aureus*. The minimum inhibitory concentration (MIC) for *B. subtilis, B.cerus, S.aureus,* and *E.coli* was 50 mg/mL when methanolic extracts of onion, neem, and bitter gourd were applied. Results at various concentrations revealed that at 110 mg/mL neem and bitter gourd extract were effective in growth restriction of *E.coli* and *S. aureus*. At 90 mg/mL, extract of onion and bitter gourd was effective against *B. subtilis,* while neem was found efficient against *B.cerus* at 130 mg/ml. By using plant extract at various temperature ranges (Table 2), it was observed that onion and neem extract successfully controlled the growth of *B.subtilis* and *E.coli*. The extract of bitter gourd was effective at 100 ^{0}C against *S. aureus*. When the plant extract was employed by changing pH (Table 3), it was found that *B. sutilis* showed restricted growth at pH 2 with the extracts of neem and bitter gourd. [24] Found very similar results for the methanolic extracts of neem and tulsi leaves against *E. coli* and *S. aureus*. Similarly, [5] showed that the methanolic extract of neem leaves gives quite big zones of inhibition for various pathogenic bacterial strains, like *P. aeruginosa*, *Citrobacter* subsp., *Klebsiella pneumoniae*, *E. coli*, *Enterococcus faecalis*, *Proteus* subsp., *Staphylococcus epidermidis*, and *S. aureus*. They suggested that Geranyl β-D-glucopyranoside was responsible for this antibacterial activity [5]; [25] tested the effect of aqueous and ethanolic onion extracts against different bacterial strains, including *E. coli*, *Salmonella* subsp., *Streptococcus pneumoniae*, *Shigella* subsp., and *S. aureus*. They reported that the zone of inhibition for *E. coli* was 23 mm, *Salmonella* spp. 20 mm, *S. pneumoniae* 20 mm, *Shigella* subsp. 21 mm, and for *Staphylococcus aureus* it was 21 mm, respectively. The results demonstrated that both ethanolic and aqueous plant extracts have good antibacterial activity [34]. [35] Describes the antibacterial activity of *A. cepa* against bacteria strains responsible for causing intestinal infection, such as *E. coli* and *Vibrio cholerae*. The onion extract under study was found to be most effective against *E. coli*. This study also revealed that onion extracts have the capability to show antibacterial activity against bacteria causing enteric infections.

[36] Conducted research on neem extract. The extract was tested against bacterial strains, i.e.,

Table 1. Effect of methanolic extract and Amoxil (500mg) at various concentrations.

Bacterial strains	Plant name	Methanolic extracts at different concentration (mg/mL)					Negative control	Positive control	*P* Value
		50	70	90	110	130			
B. subtilis	Onion	0.3±0.2	1.2±0.2	1.6±0.1	1.3±0.1	1.2±0.2	0±00	2.2±0.25	0.260
	Neem	0.9±0.1	1.2±0.1	1.3±0.2	1.2±0.1	0.9±0.1	0±00		0.016*
	bitter gourd	0.1±0.1	0.9±0.1	1.6±0.1	1.4±0.1	1±0.1	0±00		0.000*
E. coli	Onion	1.3±0.2	0.9±0.1	1.0±0.1	1.5±0.1	1.1±0.1	0±00	1.8±0.69	0.007*
	Neem	1.4±0.2	1.1±0.0	1.3±0.2	1.7±0.1	1.2±0.1	0±00		0.000*
	bitter gourd	1.2±0.2	1.3±0.0	1.3±0.2	1.5±0.1	1.2±0.1	0±00		0.013*
S. aureus	Onion	0.9±0.0	1.0±0.2	1.0±0.0	1.3±0.2	1.4±0.2	0±00	2.1±0.20	0.117
	Neem	1.1±0.1	1.2±0.1	0.9±0.1	1.8±0.1	0.7±0.2	0±00		0.000*
	bitter gourd	1.3±0.1	1.4±0.1	1.7±0.1	1.8±0.1	0.5±0.2	0±00		0.016*
B. cereus	Onion	0.9±0.1	1.2±0.2	1.2±0.2	0.9±0.1	1.1±0.1	0±00	2.1±0.08	0.178
	Neem	1.1±0.0	1.2±0.1	1.5±0.1	1.0±0.1	1.6±0.1	0±00		0.000*
	bitter gourd	0.7±0.1	1.3±0.1	1.3±0.1	0.5±0.1	1.4±0.1	0±00		0.000*

Note: *Mean ± SD (*n*=3). Values present in rows are variables. Found the P values by using ANOVA and compared with significant (*p*≤0. 05). Non-significant values are (*p*≥0. 05)

Table 2. Effect of methanolic plant extracts at various temperature range.

Bacterial strains	Plant name	Temperature (°C) range					*P* value
		20	40	60	80	100	
B. subtilis	Onion	1.5±0.5	2±.4	1±0.4	2.3±0.4	2.4±0.2	0.128
	Neem	1.5±0.2	1.7±0.2	1.0±0.2	1.4±0.2	1.7±0.1	0.002*
	bitter gourd	1.5±0.5	2±1.4	1.2±0.4	2±0.4	2±0.2	0.127
E. coli	Onion	1.1±0.2	0.8±0.1	0.7±0.1	0.9±0.0	1.3±0.0	0.021*
	Neem	1.5±0.1	1.3±0.1	1.1±0.1	1.3±0.1	1.7±0.2	0.000*
	bitter gourd	1.2±0.2	1.2±0.5	1.5±0.5	1.2±0.5	1.5±0.6	0.001*
S. aureus	Onion	0.8±0.2	0.9±0.0	0.8±0.1	1.5±0.2	1.5±0.2	0.001*
	Neem	1.1±0.1	1.0±0.1	0.8±0.1	1.5±0.2	1.6±0.1	0.000*
	bitter gourd	1±0	0±00	0±00	0±00	1.5±0.5	0.000*
B. cereus	Onion	0.8±0.1	0.9±0.1	0.8±0.2	0.9±0.1	1.7±0.0	0.000*
	Neem	0.1±0.2	2.5±1.75	2.5±1.5	4±1.5	1.3±0.75	0.000*
	bitter gourd	0.1±0.5	2.5±1.25	2.5±1.5	3±1.25	2±1.3	0.169

Note: *Mean ± SD (*n*=3). Values present in rows are variables. Found the P values by using ANOVA and compared with significant (*p*≤0. 05). Non-significant values are (*p*≥0. 05)

Micrococcus glutamicus, *Lactobacillus bulgaris*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Staphylococcus pyogenes*, *Micrococcus luteus*, *B. cereus*, *E. coli*, and *Pseudomonas aeruginosa*. The extract showed results against all test strains. Maximum inhibitory zones were observed against *Pseudomonas aeruginosa* [36].

Table 3. Effect of methanolic extracts at different pH range.

Bacterial Strains	Plant name	pH range					*P* Value
		2	5	7	9	13	
B. subtilis	Onion	1.1±0.0	1.3±0.1	1.6±0.0	1.2±0.2	1.3±0.2	.024*
	Neem	1.4±0.1	1.0±0.1	1.0±0.1	1.0±0.2	1.0±0.1	.044*
	bitter gourd	1.5±0.2	1.5±0.4	2.5±0.3	1.3±0.5	1.3±0.7	.024
E. coli	Onion	1.0±0.1	1.0±0.1	1.2±0.2	0.9±0.1	1.1±0.1	.182
	Neem	1.0±0.1	1.1±0.1	1.1±0.1	0.9±0.0	0.4±0.2	.001*
	bitter gourd	1.0±0.3	1.5±0.6	1.2±0.6	0.2±0.0	1.5±0.5	.001*
S. aureus	Onion	1.4±0.2	1.2±0.1	1.1±0.1	1.0±0.1	1.1±0.2	.059
	Neem	1.5±0.0	1.0±0.1	1.3±0.2	1.0±0.1	1.0±0.1	.003*
	bitter gourd	1.3±0.3	1.3±0.2	1.4±0.2	1.1±0.2	1.2±0.4	.217
B. cereus	Onion	1.3±0.1	1.0±0.1	1.2±0.1	1.2±0.1	1.2±0.0	.036*
	Neem	1.4±0.1	1.3±0.1	1.1±0.1	1.2±0.1	0.9±0.0	.004*
	bitter gourd	1.4±0.5	1.2±0.25	1.2±0.4	1.3±0.75	1.2±0.5	.009*

Note: *Mean ± SD (n=3). Values present in rows are variables. Found the P values by using ANOVA and compared with significant (p≤0. 05). Non-significant values are (p≥0. 05)

[37] Performed experiments on food bacterial strains and bitter gourd gave effective results against them. The MIC used there is 5mg/ml and it shows the zone of inhibition at 12 mm. It is also found that the antibacterial activity of bitter gourd at different concentrations against *E.coli* is reported to be effective in controlling the bacterial strain [38].

The zones of inhibition for the methanolic extracts of neem leaves at the 50 mg/mL and 130 mg/mL concentrations after different temperature treatments are shown in Tables 1 and 1.3. A 4 mm inhibitory zone was observed for neem methanolic extract that was treated at 80°C against B. cereus. Both neem and onion methanolic extracts show a zone of inhibition at a lower concentration of 50 mg/mL and the highest 130 mg/mL concentration by altering five different ranges of pH shown in Table 3. By comparing the means of all, using temperatures of all test bacterial strains, the P value was significant. [39] conducted a similar study by taking ethanolic extract from six plants (*Psidium guajava L., Salvia officinalis L., Ziziphusspina Christi (L.) Desf., Morus alba L.*, and *Olea europaea L.*) leaves against different bacterial strains like *S. aureus, E. coli, Pasteurella multocida*, *B. cereus*, *Salmonella* enteritidis, and *M. gallisepticum* using agar disc diffusion technique and minimal inhibitory concentration (MIC). Their results revealed that only *Psidium guajava* was found effective against *Mycoplasma gallisepticum*, while all other extracts were effective against the remaining strains under study at variable concentrations ranging from 625 to 5000 µg/mL.

The pH result showed neem is slightly alkaline in nature and onion pH is acidic in nature. Another similar study reported that the ethanolic extract of roselle showed noticeable antibacterial activity against *B. cereus, S. aureus*, *E. coli, S. enteritidis, Vibrio parahaemolyticus*, and *P. aeruginosa* while having no effect against *Candida albicans* (fungus). Only the extract of thyme and clove showed an antifungal effect against *C. albicans.* Their study concluded that plant extracts affected the gram-positive and gram-negative strains at an acidic pH, disrupting the cell membranes of selected strains [40]. According to earlier reports, water extract from various plants was found to be more effective against bacterial strains as compared with the ethanolic extract from the same plants [40]. This might be due to the higher polarity of water and the use of elevated temperature during plant extract making [41]. Combining all the results for the three extracts under investigation, including the results obtained for different temperature and pH treatments, it can be concluded that neem extract exhibits a better antibacterial potential than onion and bitter gourd methanolic extract. The collective findings from this study confirm that methanolic extracts of neem, onion, and bitter gourd possess significant antibacterial properties against a range of pathogenic bacteria. The efficacy of these extracts, as demonstrated by the inhibition zones and MIC values, highlights their potential as alternative therapeutic agents in the fight against bacterial infections. Future studies should focus on further characterizing the active constituents of these extracts and exploring their integration into conventional treatment paradigms. By harnessing the power of these natural compounds, we can potentially develop more effective strategies to combat the growing challenge of antibiotic resistance. This research lays a foundational step towards utilizing ethnobotanical resources for enhancing public health initiatives and underscores the necessity for continuous and in-depth exploration of plant-based antimicrobials.

Conclusions

The presence of various secondary metabolites with proven antimicrobial activity, such as steroids, flavonoids, alkaloids, and tannins, has been revealed by chemical prospection of selected plant extracts. In this investigation, neem, bitter gourd, and onion extract showed antibacterial activity against a number of foodborne pathogens. The methanolic plant extracts from all three extracts show a correlation when compared, and the assay to calculate the MIC has shown the efficacy of the extracts to be comparable with the selected antibiotic as a positive control. This study's findings also imply that phytochemical compounds that have antibacterial characteristics may be present in onion, bitter gourd, and neem extracts, making them valuable in the control of pathogens and bacteria causing food poisoning. The outcomes of the current study suggest that it will take a combination of approaches to combat the threat of antibiotic resistance, and the ethnobotanical approach provides the means to unlock and apply plant chemistry that is useful for the discovery of antibiotic compounds. Future research should focus on identifying the mechanisms through which these extracts combat bacterial resistance, potentially paving the way for novel therapeutic applications.

Acknowledgements

The authors extend their appreciation to the Researchers supporting project number (RSP2025R185), King Saud University, Riyadh, Saudi Arabia.

Author's Contribution

All authors contribute equally to this work.

Conflict of Interest

The authors declare no conflict of interest.

References

1. KUMAR K.S., BHOWMIK D., CHIRANJIB B., TIWARI P. Allium cepa: A traditional medicinal herb and its health benefits. Journal of Chemical and Pharmaceutical Research, **2** (1), 283, **2010**.
2. ASADI-SAMANI M., KAFASH-FARKHAD N., AZIMI N., FASIHI A., ALINIA-AHANDANI E., RAFIEIAN-KOPAEI M. Medicinal plants with hepatoprotective activity in Iranian folk medicine. Asian Pacific Journal of Tropical Biomedicine, **5** (2), 146, **2015**.
3. SULTANA S., ASIF H.M., AKHTAR N., WAQAS M., REHMAN S.U. Comprehensive Review on Ethanobotanical Uses, Phytochemistry and Pharmacological Properties of Melia azedarach Linn. Asian Journal of Pharmaceutical Research and Health Care, 26, **2014**.
4. AKRAM N.A., SALEEM M.H., SHAFIQ S., NAZ H., FARID-UL-HAQ M., ALI B., SHAFIQ F., IQBAL M., JAREMKO M., QURESHI K.A. Phytoextracts as crop biostimulants and natural protective agents—a critical review. Sustainability, **14** (21), 14498, **2022**.
5. PATIL P., PATIL S., MANE A., VERMA S. Antidiabetic activity of alcoholic extract of neem (Azadirachta indica) root bark. National Journal of Physiology, Pharmacy and Pharmacology, **3** (2), 142, **2013**.
6. LIAUW M.Y., NATAN F., WIDIYANTI P., IKASARI D., INDRASWATI N., SOETAREDJO F. Extraction of neem oil (Azadirachta indica A. Juss) using n-hexane and ethanol: studies of oil quality, kinetic and thermodynamic. ARPN Journal of Engineering and Applied Sciences, **3** (3), 49, **2008**.
7. RAUT R.R., SAWANT A.R., JAMGE B.B. Antimicrobial activity of Azadirachta indica (Neem) against pathogenic microorganisms. Journal of Academia and Industrial Research, **3** (7), 327, **2014**.
8. PATIL S.M., SHIRAHATTI P.S., RAMU R. Azadirachta indica A. Juss (neem) against diabetes mellitus: A critical review on its phytochemistry, pharmacology, and toxicology. Journal of Pharmacy and Pharmacology, **74** (5), 681, **2022**.
9. DEBJIT B., JITENDER Y., TRIPATHI K., KUMAR K.S. Herbal remedies of Azadirachta indica and its medicinal application. Journal of Chemical and Pharmaceutical Research, **2** (1), 62, **2010**.
10. AHMAD S., MAQBOOL A., SRIVASTAVA A., GOGOL S. Biological detail and therapeutic effect of Azadirachta indica (neem tree) products-a review. Evidence Based Medical Healthcare, **6** (22), 1607, **2019**.
11. BANDYOPADHYAY U., BISWAS K., CHATTERJEE R., BANDYOPADHYAY D., CHATTOPADHYAY I., GANGULY C.K., CHAKRABORTY T., BHATTACHARYA K., BANERJEE R.K. Gastroprotective effect of Neem (Azadirachta indica) bark extract: Possible involvement of H+-K+-ATPase inhibition and scavenging of hydroxyl radical. Life Sciences, **71** (24), 2845, **2002**.
12. MBAH A., UDEINYA I., SHU E., CHIJIOKE C., NUBILA T., UDEINYA F., MUOBUIKE A., MMUOBIERI A., OBIOMA M.S. Fractionated neem leaf extract is safe and increases CD4+ cell levels in HIV/AIDS patients. American Journal of Therapeutics, **14** (4), 369, **2007**.
13. ALBISHI T., JOHN J.A., AL-KHALIFA A.S., SHAHIDI F. Antioxidative phenolic constituents of skins of onion varieties and their activities. Journal of Functional Foods, **5** (3), 1191, **2013**.
14. OUYANG H., HOU K., PENG W., LIU Z., DENG H. Antioxidant and xanthine oxidase inhibitory activities of total polyphenols from onion. Saudi Journal of Biological Sciences, **25** (7), 1509, **2018**.
15. JAKARIA M., AZAM S., CHO D.-Y., HAQUE M.E., KIM I.-S., CHOI D.-K. The methanol extract of Allium cepa L. protects inflammatory markers in LPS-induced BV-2 microglial cells and upregulates the antiapoptotic gene and antioxidant enzymes in N27-A cells. Antioxidants, **8** (9), 348, **2019**.

16. LOREDANA L., GIUSEPPINA A., FILOMENA N., FLORINDA F., MARISA D. M., DONATELLA A. Biochemical, antioxidant properties and antimicrobial activity of different onion varieties in the Mediterranean area. Journal of Food Measurement and Characterization, **13**, 1232, **2019**.
17. TSUBOKI J., FUJIWARA Y., HORLAD H., SHIRAISHI D., NOHARA T., TAYAMA S., MOTOHARA T., SAITO Y., IKEDA T., TAKAISHI K. Onionin A inhibits ovarian cancer progression by suppressing cancer cell proliferation and the protumour function of macrophages. Scientific Reports, **6** (1), 29588, **2016**.
18. SALAMI M., TAMTAJI O.R., MOHAMMADIFAR M., TALAEI S.A., TAMEH A.A., ABED A.R., SHIRKHODA S., DADGOSTAR E., TAGHIZADEH M. Neuroprotective effects of Onion (Allium cepa) Ethanolic Extract on Animal Model of Parkinson's Disease Induced by 6-hydroxydopamine: A Behavioral, Biochemical, and Histological Study. Gazi Medical Journal, **31** (1), **2020**.
19. LEE J.-S., CHA Y.-J., LEE K.-H., YIM J.-E. Onion peel extract reduces the percentage of body fat in overweight and obese subjects: a 12-week, randomized, double-blind, placebo-controlled study. Nutrition Research and Practice, **10** (2), 175, **2016**.
20. MALLIKARJUNA K., TOMAR B.S., MANGAL M., SINGH N., SINGH D., KUMAR S., TOMER A., SINGH B., JAT G.S. Genetic diversity and population structure analyses in bitter gourd (Momordica charantia L.) based on agro-morphological and microsatellite markers. Plants, **12** (19), 3512, **2023**.
21. GAYATHRY K., JOHN J.A. A comprehensive review on bitter gourd (Momordica charantia L.) as a gold mine of functional bioactive components for therapeutic foods. Food Production, Processing and Nutrition, **4** (1), 10, **2022**.
22. ROJAS R., BUSTAMANTE B., BAUER J., FERNÁNDEZ I., ALBÁN J., LOCK O. Antimicrobial activity of selected Peruvian medicinal plants. Journal of Ethnopharmacology, **88** (2-3), 199, **2003**.
23. BENKEBLIA N. Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). LWT-Food Science and Technology, **37** (2), 263, **2004**.
24. TANAKA H., SATO M., FUJIWARA S., HIRATA M., ETOH H., TAKEUCHI H. Antibacterial activity of isoflavonoids isolated from Erythrina variegata against methicillin-resistant Staphylococcus aureus. Letters in Applied Microbiology, **35** (6), 494, **2002**.
25. BISWAS K., CHATTOPADHYAY I., BANERJEE R.K., BANDYOPADHYAY U. Biological activities and medicinal properties of neem (Azadirachta indica). Current Science, 1336, **2002**.
26. MOHAMMED H.A., OMER A. Antibacterial activity of Azadirachta indica (Neem) leaf extract against bacterial pathogens in Sudan. American Journal of Research Communication, **3** (5), 246, **2015**.
27. SHARMA J., GAIROLA S., SHARMA Y.P., GAUR R. Ethnomedicinal plants used to treat skin diseases by Tharu community of district Udham Singh Nagar, Uttarakhand, India. Journal of Ethnopharmacology, **158**, 140, **2014**.
28. KABRAH M.A.M., FAIDAH H.S., ASHSHI A.M., TURKISTANI M.S.A. Antibacterial effect of onion. Scholars Journal of Applied Medical Sciences, **4** (11D), 4128, **2016**.
29. COSTA J.G.M., NASCIMENTO E.M., CAMPOS A.R., RODRIGUES F.F. Antibacterial activity of Momordica charantia (Curcubitaceae) extracts and fractions. Journal of Basic and Clinical Pharmacy, **2** (1), 45, **2010**.
30. KUMARI P.D., SHENOY S.M., KHIJMATGAR S., CHOWDHURY A., LYNCH E., CHOWDHURY C.R. Antibacterial activity of new atraumatic restorative treatment materials incorporated with Azadirachta indica (Neem) against Streptococcus mutans. Journal of Oral Biology and Craniofacial Research, **9** (4), 321, **2019**.
31. KUMAR V., CHAKRABORTY A., KAUR M., PANDEY S., JENA M.K. Comparative study on antimicrobial activity of tulsi (Ocimum sanctum) and neem (Azadirachta indica) methanol extract. Asian Journal of Pharmaceutical and Clinical Research, 514, **2018**.
32. ALTAYB H.N., YASSIN N.F., HOSAWI S., KAZMI I. In-vitro and in-silico antibacterial activity of Azadirachta indica (Neem), methanolic extract, and identification of Beta. d-Mannofuranoside as a promising antibacterial agent. BMC Plant Biology, **22** (1), 262, **2022**.
33. OYEBODE J., FAJILADE T. Antibacterial activities of aqueous and ethanolic extract of Allium cepa (onion bulb) against some selected pathogenic microorganisms. International Journal of Scientific and Research Publications, **4**, 2250, **2014**.
34. INDUJA M., GEETHA R. Antimicrobial activity of Allium cepa against bacteria causing enteric infection. Drug Invention Today, **10** (12), **2018**.
35. RAJASEKARAN C. Investigations on antibacterial activity of leaf extracts of Azadirachta indica A. Juss (Meliaceae): a traditional medicinal plant of India. Ethnobotanical Leaflets, **2008** (1), 161, **2008**.
36. MASITHOH D., KUSDARWATI R., HANDIJATNO D. Antibacterial activity of bitter gourd (Momordica charantia L.) leaf extract against Aeromonas hydrophila. IOP Publishing, **236,** 012096, **2019**.
37. ADEYEMI A.O., OLUWASEGUN A.D. Antibacterial Activity of Aqueous Extract of Momordica charantia and Terminalia catappa on Multidrug Resistant Invasive Escherichia coli isolated in Ready-to-Eat (RTE) Foods from Ekiti State: Momordica charantia and Terminalia catappa Effect on E. coli of food. Biological Sciences-PJSIR, **62** (3), 183, **2019**.
38. HEMEG H.A., MOUSSA I.M., IBRAHIM S., DAWOUD T.M., ALHAJI J.H., MUBARAK A.S., KABLI S.A., ALSUBKI R.A., TAWFIK A.M., MAROUF S.A. Antimicrobial effect of different herbal plant extracts against different microbial population. Saudi Journal of Biological Sciences, **27** (12), 3221, **2020**.
39. GONELIMALI F.D., LIN J., MIAO W., XUAN J., CHARLES F., CHEN M., HATAB S.R. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Frontiers in Microbiology, **9**, 389103, **2018**.
40. CALEJA C., BARROS L., ANTONIO A.L., CAROCHO M., OLIVEIRA M.B.P., FERREIRA I.C. Fortification of yogurts with different antioxidant preservatives: A comparative study between natural and synthetic additives. Food Chemistry, **210**, 262, **2016**.
41. DHANANI T., SHAH S., GAJBHIYE N., KUMAR S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. Arabian Journal of Chemistry, **10**, S1193, **2017**.