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Determination of antibacterial and antioxidant potential of organic crude extracts from *Malus domestica*, *Cinnamomum verum* and *Trachyspermum ammi*

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Plants are the rich source of biologically active compounds which can be obliging against various pathogenic microorganisms and cancerous diseases. The current study evaluated the antibacterial potential of aqueous, methanol, ethanol, and acetone extracts of *Malus domestica* (apple), *Cinnamomum verum* (cinnamon) and *Trachyspermum ammi* (ajwain) *via* agar well diffusion methods and minimum inhibitory concentration (MIC) in (mm) against *Staphylococcus aureus* (ATCC 25923) and *Salmonella typhi* (ATCC 19430). The antioxidant properties including total phenolic content (TPC), total flavonoid content (TFC), DPPH and reducing power was determined by UV/VIS spectrophotometery and all the results interpreted through one way ANOVA (STATISTICA). In the results, methanolic and acetonic extracts of *C. verum* has shown maximum zone of inhibition (22.3 ± 0.58 mm) against *S. aureus* while for *C. verum* and *T. ammi*, ethanolic extracts has expressed the maximum zone of inhibition (22.3 ± 0.58 mm) against *S. typhi* and for *M. domestica* the methanolic extracts has exhibited highest zone of inhibition (18 ± 0.56 mm) among all other extracts of *M. domestica*. The MIC values were comparable with antimicrobial activity. Among the antioxidant activity analysis, the highest level of TPC has observed in aqueous extract of *M. domestica* 72.15 ± 1.80 mg GAE/g, while highest TFC was observed in methanolic extracts of *M. domestica* 15.62 ± 0.25 µg CE/g. The DPPH assay showed maximum percentage inhibition 123% in the methanolic extract of *M. domestica*, while highest reducing potential 13.42 ± 1.15 nm was observed in aqueous extract of *C. verum*. This study has compared three potential medicinal plants with biological active and eco-friendly components which play crucial role in therapeutics.

Keywords Plant extracts, Antimicrobial, Antioxidant

The use of plants is very common worldwide for the therapeutic purposes[1]. The medicines which have active biological compounds extracted from plant source[2]are gaining popularity these days, owing to growing concerns about potentially dangerous synthetic chemicals[3,4]. Natural products have long been recognized as important components in the production of contemporary drugs, particularly antibacterial and antitumor medicines[5,6]. Almost all plants have active antibacterial potential but at different level[7,8]. Some plants are rich in highly powerful antibacterial compounds[9]but some plants have less active compounds[10]. These active compounds are present in the form of metabolites[11,12].

The metabolites of plants are some of the most powerful agents in various diagnostics and therapeutic processes[13–15]. These metabolites are of two types i.e. primary metabolites and secondary metabolites[16]. The solvent organic extracts of different plants contain different secondary metabolites including terpenoids,

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flavonoids, alkaloids, and many other phenolic compounds[17–19]. The secondary metabolites of plants can be extracted through different types of solvents including polar solvents which are organic in nature, solvents with intermediate polarity like methanol, ethanol, acetone etc., and low polarity solvents like hexane. The plants extracts with different metabolites can also have distinct antibacterial and other biological properties[20–22].

The organic extracts of different plant's species *Malus domestica*, *Cinnamomum verum* and *Trachyspermum ammi* have been investigated at experimental level since many years. The antioxidant and antibacterial properties of *Malus domestica* is much known from past few years and it is reported that *Malus domestica* inhibits the oxidative stress and has abundant therapeutic benefits. The antioxidant and antibacterial potential of *Malus domestica* is attributed to its excellent phytochemicals including phenolic and flavonoid compounds. These bioactive components include catechins, quercetin, and chlorogenic acids which mutually contribute to the antioxidant potential of *Malus domestica*. Different components of *Malus domestica*extract have various medicinal properties but quercetin is specially used to treat inflammation and different cardiovascular disorders[25–27]. The *Cinnamomum verum*has potent anti-allergic, anti-pyretic[28,29], antioxidant, anti-inflammatory, and antibacterial compounds which showed beneficial role in the current scientific research. Cinnamaldehyde is the key component present in *Cinnamomum verum*and responsible for not only produce distinct flavor but also exhibit anti-inflammatory and antibacterial potential[30–33].

Trachyspermum ammi belongs to the *Apiaceae*family and one of the aromatic seed spices[34–36]. It has medicinal properties and mostly used as a digestive stimulant. The organic solvents of *Trachyspermum ammi* contain high proportion of phenolic compounds which has great antibacterial and anti-oxidant abilities. Various antioxidants present in *Trachyspermum ammi*but thymol is the most potent bioactive component responsible for strong antioxidant properties[37–39].

The antibacterial compounds of plant extracts can degrade microorganisms via different ways i.e. inhibiting DNA gyrase, protein biosynthesis, efflux pump, and disruption of cell membrane (Fig. 1)[40–42]. Hence, they can prove to be a potent agent against lots of bacterial infections and resistance. The main aim of the current study was to compare the antibacterial and antioxidant potential of selected plant's extracts i.e. *Malus domestica*, *Cinnamomum verum*, and *Trachyspermum ammi* using different organic solvents and to determine the efficacy of these solvents to extract various bioactive components including antibacterial, phenolic and flavonoid compounds. Furthermore, the bioactive components from these plant extracts were subjected to assess the antibacterial and antioxidant potential to combat broad spectrum pathogenic microorganisms and reactive oxygen species (ROS), with the goal of providing safe and natural alternative to chemical based drugs.

Materials and methods

The chemicals and reagents were obtained from Sigma Aldrich and Merck Germany. The instruments used in this study were obtained from Omron Japan, Dalton Japan, Sanyo, Germany, Ohyo Japan, Gilson U.S.A,

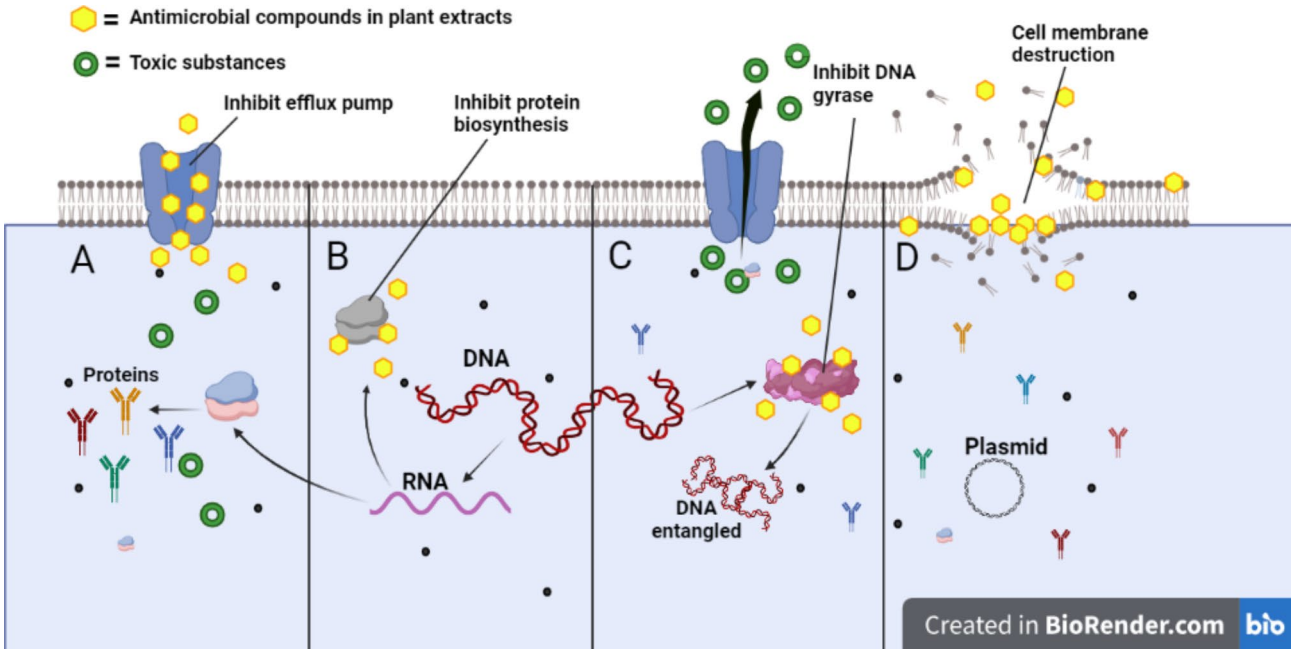

Fig. 1. Degradation pathways of antimicrobial compounds extracted from plant source. (**A**) antibacterial compounds in plants extracts can inhibit efflux pump, which remove toxic substances from bacterial cell to survive inside host body (**B**) inhibit protein synthesis by restricting the activity of ribosomes[23](**C**) DNA gyrase help to prevent DNA entanglement after replication, antibacterial compounds inhibit the working of DNA gyrase cause entangled DNA (**D**) cell membrane can be destructed through antibacterial compounds cause release of cytoplasmic content to environment[24].

Gallenkamp UK, Linbro, USA, Dawlance Pakistan, Bio Tek, U.S.A. The *M. domestica*, *C. verum* and *T. ammi* were purchased from local market of Lahore and maintained at 4 °C until processing.

Preparation of plant extracts

All the plants were obtained from Local market of Lahore, Pakistan. The *Cinnamomum verum*bark was washed, dried, and crushed into fine powder. The extraction was done through conventional method by using the guidelines of[43]. The obtained fine powder was heated with hot water by ratio (1:3 w/w) at 60 °C for 3 h. Then mixture was filtered, dried and concentrated under reduce pressure at rotatory evaporator (60 °C). The equal ratio (1:1 w/v) of dried powder was added separately in four different organic extracts including water, methanol, ethanol and acetone and kept overnight at 4 °C to precipitate the sample. After precipitation, samples were centrifuged at 5000×g for 20 min. The extracts were weighed and stored in tightly sealed amber-colored glass vials, to prevent from light exposure, at 4°C. Same protocol was followed to prepare extracts of *Malus domestica* fruit and *Trachyspermum ammi* seeds. The *Malus domestica* fruit was washed thoroughly, crushed and dried for several days to remove moisture while ajwain seeds were washed and grinded to fine powder.

Antibacterial activity of plant extracts

The pure bacterial cultures of gram-positive strain *Staphylococcus aureus* and gram-negative strain *Salmonella typhi* were prepared through nutrient agar 2.8% and nutrient broth 1.3% and stored at 4°C. The antibacterial activity of plant extracts was determined through agar well diffusion method. The nutrient agar was kept to solidify and then pure bacterial culture was poured over the agar, 5 mm wells were created into the solid agar and sterilized forceps were used to remove the well plugs. The 25 mg/mL of plant extracts were poured into the wells and incubated at 37°C for 24 h under aerobic conditions to obtain 10^6CFU or spores/mL[44]. The activity index (AI) of all plant extracts was also evaluated in order to determine the strength of antibacterial activity against broad spectrum microorganisms. The AI was calculated by using the formula (1) given below[45]:

$$Activity\ index(AI) = \frac{\text{Diameter of zone of inhibition of plant extract (mm)}}{\text{Diameter of zone of inhibition of standard (mm)}} \quad (1)$$

Minimum inhibitory concentration (MIC) of plant extracts

The minimum inhibitory concentration (MIC) of different plant extract was determined on the basis of their antimicrobial potential. In this study, the methanolic, ethanolic, and acetonic extract of *C. verum*, methanolic and ethanolic extract of *T. ammi*, and methanolic extract of *M. domestica*was selected as they showed maximum diameter of zones of inhibition with respect to other plant extracts. The micro dilution plates were used to measure the minimum inhibitory concentration of plant extracts by using the guidelines with slide modifications given by[46] and 100 mL of nutrient broth were poured into the 96 wells. Using two fold dilution method, added 100 μL of sample to the first well. Then added 20 μL of the given bacterial culture to each well and incubate for 24 h at 37 °C. The absorbance was measured at 620 nm.

Antioxidant activity of plant extracts

Different antioxidant assays were performed to determine the antioxidant potential of these plant extracts.

Total phenolic and flavonoids contents

The total phenolics contents (TPC) were determined using Folin-Ciocalteu (FC) reagent. Plants extract (1mL; 0.001 g/mL) was mixed with FC reagent and optical density of blue colored complex was taken at 765 nm after 1 h using 96 well Microplate reader, Model sμ-QuantTM, from BioTek, USA. All determinations were performed in triplicates. Gallic acid was used as the standard (Fig. 2). Total phenolic contents of studied plants extract was quantified as Gallic acid equivalents (GAE) and calculated using the formula (2)[47].

$$TPC\ in\ 1g\ of\ plant\ extract\ as\ mg\ GAE = \frac{GAE\ from\ standard\ curve\ \left(\frac{mg}{mL}\right) \times\ extract\ volume\ (mL)}{Extract\ weight\ (g)} \quad (2)$$

The total flavonoids contents (TFC) were assayed and briefly plants extract (0.5 mL) was added to 2 mL distilled water and 0.15 mL of 5% $NaNO_2$ solution and incubated for 6 min. After adding 0.15mL of 10% $AlCl_3$ the mixture was incubated for 6 min followed by the addition of 4% NaOH solution and methanol to make the final volume up to 5 mL. Optical density of the reaction mixture was taken at 510 nm after 15 min incubation. Catechin was used as standard (Fig. 3). Total flavonoid contents (TFC) of extracts were quantified as μg catechin equivalents per gram of extract[47].

DPPH scavenging assay

Radical scavenging potential of plant extracts was estimated through DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay. For this, plants extract (3mL) methanol solution was mixed with 1 mL of 0.004% DPPH in and kept under dark environment for 30 min. The optical density at 517 nm was measured which is inversely related to radical scavenging activity. The reagent solution without plants extract was used as procedural control[47]. The percentage inhibition of plant extracts was calculated by using formula (3);

$$DPPH\ inhibition(\%) = \frac{Blank\ absorbance\ (Ao) - Sample\ absorbance\ (A1)\ \times\ 100}{Blank\ absorbance\ (Ao)} \quad (3)$$

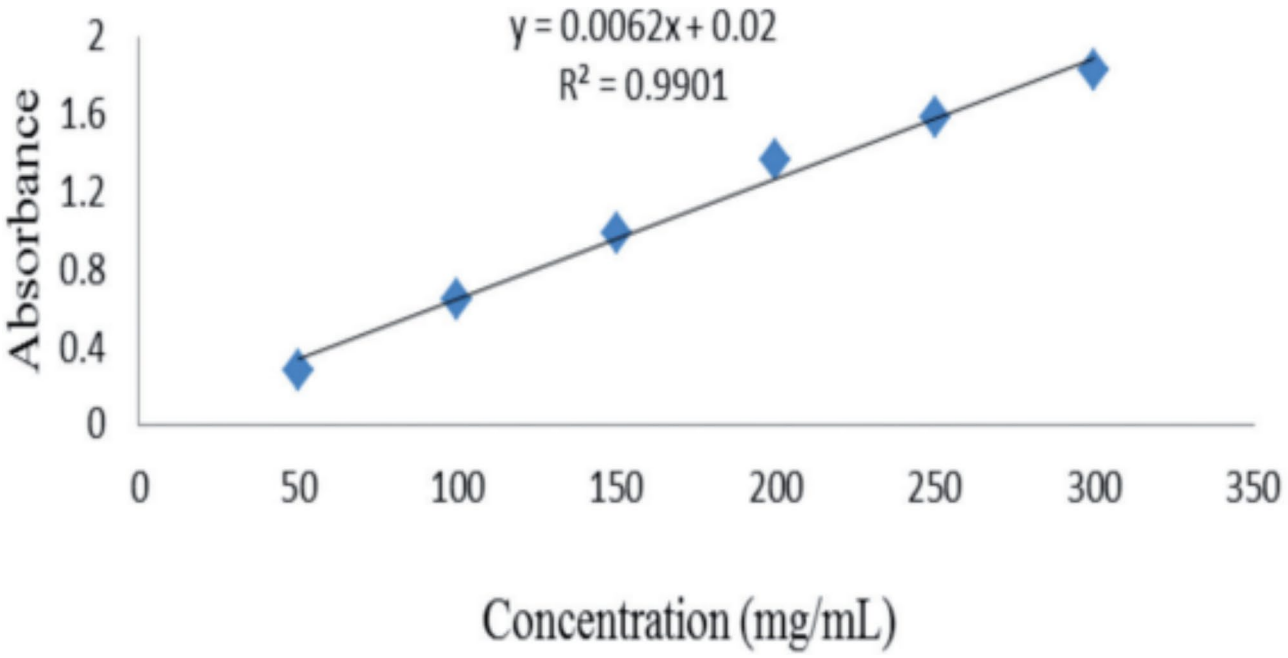

Fig. 2. Standard curve of Gallic acid for TPC[47].

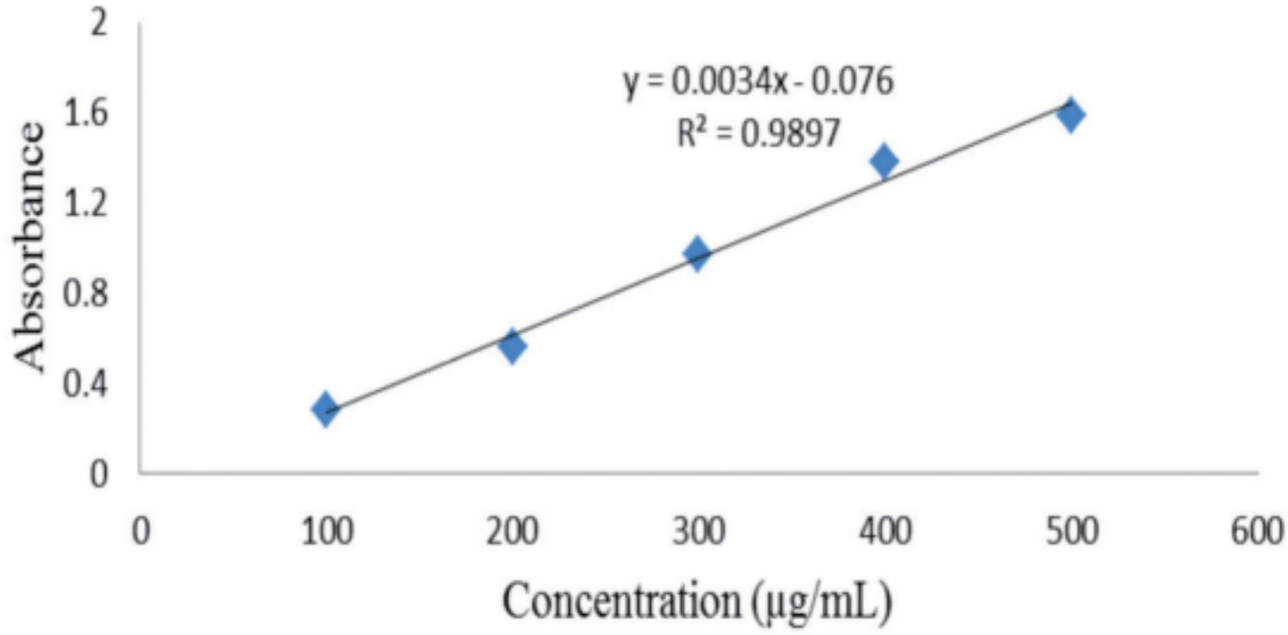

Fig. 3. Standard curve of Catechin for TFC[47].

Reducing power measurement

The ferric reducing antioxidant potential (FRAP) assay based on the principle of direct electron donation reducing the $Fe^{3+}(CN)_6$ to $Fe^{2+}(CN)_6$was used for the measurement of reducing potential of plant extracts. The reaction mixture contained 1 mL of plant extracts, phosphate buffer (2.5 mL 0.2 M; pH 6.6) and 1% potassium ferricyanide (2.5 mL). After 20 min incubation at 50 °C, stopped the reaction by adding 2.5 mL TCA (1% w/v) following centrifugation at 3000 rpm for 10 min. Supernatant (2.5mL) was added to 2.5 mL distilled water and 0.5 mL ferric chloride (0.1% w/v) and the optical density was taken at 700 nm[47].

Statistical analysis

All the experiments were employed three times and all evaluations were accomplished in triplicates ($n = 3$). The obtained results were statistically interpreted through GraphPad Prism statistical software, version 5. The

Solvents (mL)	M. domestica (g)	C. verum (g)	T. ammi (g)
Distilled water	1.50 ± 0.02	1.50 ± 0.01	1.81 ± 0.04
Ethanol	1.11 ± 0.01	1.79 ± 0.01	1.70 ± 0.01
Methanol	1.00 ± 0.03	1.85 ± 0.03	1.50 ± 0.02
Acetone	1.31 ± 0.04	1.63 ± 0.02	1.35 ± 0.01

Table 1. Amount of extracts of *Malus domestica*, *Cinnamomum verum* and *Trachyspermum ammi* in organic solvents.

Samples	Organic extracts	Zone of inhibition (mm ± SD)	
		S. aureus	S. typhi
C. verum	Aqueous	10.3 ± 0.58	10.6 ± 1.15
	Methanolic	22.3 ± 0.58	21.3 ± 1.15
	Ethanolic	18 ± 0.01	22.3 ± 0.58
	Acetonic	22.3 ± 0.58	23 ± 0.01
T. ammi	Aqueous	12 ± 0.58	11 ± 0.55
	Methanolic	20.3 ± 0.58	15.3 ± 0.59
	Ethanolic	20 ± 0.01	22.3 ± 0.58
	Acetonic	14 ± 0.01	15 ± 0.7
M. domestica	Aqueous	9 ± 1.69	11 ± 1.15
	Methanolic	18 ± 0.56	18 ± 0.58
	Ethanolic	14 ± 0.01	13 ± 0.01
	Acetonic	12.5 ± 0.58	11 ± 0.58
Rifampicin		22.5 ± 1.91	23.75 ± 1.70
DMSO		–	–

Table 2. Representation of mean value of zone of inhibition (mm ± SD) of *C. verum*, *T. ammi*, and *M. domestica* against *Staphylococcus aureus* and *Salmonella typhi* where (-): no/less activity.

antibacterial and antioxidant properties of plant extracts were interpreted through one way ANOVA and results were expressed as mean ± standard deviation.

Results

Organic extracts of plants

The plant extraction was carried out with four organic solvents. Due to difference in polarity, each organic solvent has different level of extract (Table 1). The *M. domestica* has given high amount of organic extract in distilled aqueous, acetone, ethanol, methanol while *C. verum* contained appropriate order of decreasing content i.e. methanol, ethanol, acetone, distilled water. *T. ammi* has high amount of organic extract in distilled water and then order decreased down to ethanol, methanol, and acetone.

Antibacterial activity of plants extracts

The different organic extracts of *Cinnamomum verum*, *Trachyspermum ammi* and *Malus domestica* showed antibacterial activity more or less against *Staphylococcus aureus* and *Salmonella typhi.* The highest zone of inhibition was observed for the methanol extract 22.3 ± 0.58 mm and acetone extract 22.3 ± 0.58 mm of *Cinnamomum verum* against *Staphylococcus aureus* followed by ethanol extract 18 ± 0.01 mm and aqueous extract 10.3 ± 0.58 mm whereas acetone extract of *Cinnamomum verum* showed highest zone against *Salmonella typhi* 23 ± 0.01 mm, followed by ethanol extract 22.3 ± 0.58 mm, methanol extract 21.3 ± 1.15 and aqueous extract 10.6 ± 1.15 mm (Table 2).

The highest zone of inhibition through *Trachyspermum ammi* was observed through methanol extract 20.3 ± 0.58 mm and ethanol extract 20 ± 0.01 mm against *Staphylococcus aureus* and least zone of inhibition was obtained from aqueous extract 12 ± 0.58 mm. For *Salmonella typhi*, the highest zone of inhibition 22.3 ± 0.58 mm was observed through ethanol extract of *Trachyspermum ammi* lowered down to methanol extract 15.3 ± 0.59 mm, acetone extract 15 ± 0.7 mm and aqueous extract 11 ± 0.55 mm. The antibacterial activity of *Malus domestica* was also determined and the highest zone of inhibition was observed against both bacterial strains *S. aureus* and *S. typhi* from methanol extract 18 ± 0.01 mm and lowest zone of inhibition was showed by aqueous extract 9 ± 1.69 against *S. aureus* and 11 ± 1.15 mm against *S. typhi.* The *Malus domestica* is the most useful natural thing in many medicinal purposes due to its potential metabolites and its active antibacterial potential to degrade the pathogenic microorganisms (Table 2). These results showed approximately similar antibacterial potential of plant extracts as compared to standard antibiotic i.e. Rifampicin. Hence it emphasizes the substitution of chemical based drugs with natural and plant derived antibacterial agents, offering a safe and

ecofriendly approach to treat various infectious diseases while reducing the risks of side effects comprising conventional antibiotics.

The activity index of each plant's extract was measured in order to determine the strength of plant extracts with respect to standard antibiotics i.e. Rifampicin. The ratio provides powerful insight about the efficacy of plant extracts to inhibit bacterial growth in comparison to standards. The Table 3 demonstrates the effective activity index of all plant extracts and highest AI was obtained from methanol extract of *Cinnamomum verum* 0.99 mm against *S. aureus*, methanol and acetone extracts (0.99 mm and 0.96 mm, respectively) against *S. typhi* while least AI was obtained from aqueous extract 0.4 mm against both bacterial strains. For *Trachyspermum ammi*, maximum AI was obtained from methanol extracts of 0.90 mm against *S. aureus* and ethanol extract 0.93 mm against *S. typhi* while least AI was obtained from aqueous extract against both strains. In case of *Malus domestica*, methanol extract showed maximum AI (0.81 mm) against both strains while least AI was showed by aqueous extract against *S. aureus* 0.40 mm and *S. typhi* 0.49 mm, acetonic extract 0.49 mm.

Minimum inhibitory concentration (MIC) of combined extracts of plants

The minimum inhibitory concentration (MIC) was also evaluated in order to determine the actual practical concentration of plant extracts beneficial for the therapeutic applications. For *C. verum*, the minimum inhibitory concentration of methanol extract against *S. aureus* 3.125 mg/mL while against *S. typhi* 6.25 mg/mL. Then ethanol extract showed minimum inhibitory concentration at 12.5 mg/mL against *S. typhi* but this concentration was further minimized to 3.125 mg/mL against *S. aureus*. The acetone extract of *C.* verum also showed minimum inhibitory effect at 3.125 mg/mL against *S.* aureus while 6.25 mg/mL against *S.* typhi. For *T. ammi*, the minimum inhibitory concentration of methanol extract was 12.5 mg/mL against *S. aureus* and 3.25 mg/mL against *S. typhi*. The ethanol extract of *T. ammi* showed minimum inhibitory effect at 3.125 mg/mL against *S. aureus* while 12.5 mg/mL against *S. typhi*. The antimicrobial potential of *M. domestica* showed very less effects against both bacterial strains and only considerable results were noted in methanolic extracts of *M. domestica*. Hence, the methanol extract of *M. domestica* was 12.5 mg/mL against *S. aureus* and *S. typhi* (Table 4).

Antioxidant activity of plant extracts

In aerobic and anaerobic organisms, reactive oxygen species (ROS) are continuously produced. In the absence of an effective and antioxidative defense system, an individual may suffer adverse health effects such as fitness problems. The organic extracts of *M. domestica*, *C. verum* and *T. ammi* showed antioxidant activities with different ranges. .

Total phenolic content (TPC) The TPC of plant extracts was determined in order to evaluate the antioxidant properties comprising medicinal plants. These compounds are famous due to their hydrogen donating ability to neutralize highly oxidized cellular environment. The excessive production of reactive oxygen species (oxygen free radicals) leads to cellular damage. These phenolic compounds donate their hydrogen atom which react with oxygen free radicals and replace them into hydroxyls (OH). The TPC was measured *via* Folin-Ciocalteu (FC) reagent. The organic extract of *M. domestica* had TPC in range (64.50–72.15 μg GAE/g). Aqueous *M. domestica* extracts had the highest concentration 72.15 μg GAE/g, whereas the ethanol extracts had the lowest concentration 64.50 μg GAE/g. The TPC values of organic extract of *C. verum* were ranging from (3.45–21.0 μg GAE/g). The maximum concentration was observed in methanol extract of *C. verum* 21.0 μg GAE/g and minimum concentration was observed in acetone extracts of *C. verum* 3.45 mg/g. The TPC values of organic extract of *T. ammi*

Samples	Organic extracts	Activity index (mm)	
		S. aureus	S. typhi
C. verum	Aqueous	0.45	0.44
	Methanolic	0.99	0.89
	Ethanolic	0.8	0.93
	Acetonic	0.99	0.96
T. ammi	Aqueous	0.54	0.49
	Methanolic	0.90	0.64
	Ethanolic	0.88	0.93
	Acetonic	0.62	0.63
M. domestica	Aqueous	0.40	0.49
	Methanolic	0.81	0.81
	Ethanolic	0.62	0.58
	Acetonic	0.51	0.49
Rifampicin		1	1
DMSO		–	–

Table 3. Activity index (mm) of *C. verum*, *T. ammi* and *M. domestica* against *Staphylococcus aureus* and *Salmonella typhi*.

Sample	Organic extracts	Concentration (mg/mL)	Minimum inhibitory concentration (MIC) (µg/µL)	
			S. aureus	S. typhi
C. verum	Methanol extract	25	16 ± 0.03^{a}	15.3 ± 0.03^{a}
		12.5	13 ± 0.01^{b}	12.3 ± 0.02^{b}
		6.25	9.6 ± 0.01^{c}	11 ± 0.09^{c}
		3.125	8 ± 0.06^{d}	–
		1.56	–	–
		0.78	–	–
	Ethanol extract	25	16 ± 0.01^{a}	12 ± 0.02^{a}
		12.5	13 ± 0.05^{b}	10 ± 0.01^{b}
		6.25	9 ± 0.01^{c}	–
		3.125	8 ± 0.01^{d}	–
		1.56	–	–
		0.78	–	–
	Acetone extract	25	18.3 ± 0.01^{a}	16 ± 0.01^{a}
		12.5	15.6 ± 0.03^{b}	14 ± 0.01^{b}
		6.25	11 ± 0.01^{c}	10 ± 0.02^{c}
		3.125	9 ± 0.01^{d}	–
		1.56	–	–
		0.78	–	–
T. ammi	Methanol extract	25	11.3 ± 0.02^{a}	18.03 ± 0.02^{a}
		12.5	9 ± 0.02^{a}	16.15 ± 0.02^{a}
		6.25	–	13 ± 0.03^{b}
		3.125	–	11 ± 0.02^{c}
		1.56	–	–
		0.78	–	–
	Ethanol extract	25	16 ± 0.01^{a}	12 ± 0.03^{a}
		12.5	13 ± 0.05^{b}	10 ± 0.01^{b}
		6.25	9 ± 0.01^{c}	–
		3.125	8 ± 0.02^{d}	–
		1.56	–	–
		0.78	–	–
M. domestica	Methanol extract	25	12 ± 0.03^{a}	12.4 ± 0.01^{b}
		12.5	9 ± 0.02^{b}	8.7 ± 0.04^{c}
		6.25	–	–
		3.125	–	–
		1.56	–	–
		0.78	–	–
Rifampicin			6.25 ± 0	5.56 ± 0
DMSO			–	–

Table 4. Minimum inhibitory concentration (MIC) of plants extracts *C. verum*, *T. ammi* and *M. domestica* in different organic solvents against *Staphylococcus aureus* and *Salmonella typhi*, means carrying different superscripted alphabets (a-d) were significantly different among means, where (-): no/less activity. Values are expressed as mean ± S.D (Key: Rifampicin = positive control, DMSO = Negative control).

ranging from 0.197 to 0.891 µg GAE/g. Methanol extract of *T. ammi* in displayed the highest concentration 0.89 µg GAE/g and water extract of *T. ammi* displayed the lowest 0.197 µg GAE/g (Table 5).

Total flavonoids content (TFC) The organic extract of *M. domestica* had TFC values ranging from 12.50 to 15.62 µg CE/g. The *M. domestica* had the highest concentration in methanol extracts 15.62 µg CE/g while the lowest concentration was found in acetone extracts 12.50 µg CE/g. The organic extract of *C. verum* had TFC in range 3.124–8.56 µg CE/g and maximum concentration was observed in methanol extract of *C. verum* 8.56 µg CE/g while minimum concentration was 3.124 µg CE/g in ethanolic extract. TFC values range from 0.038 to 0.055 µg CE/g in organic extract of *T. ammi*. The *T. ammi* extract in ethanol showed highest concentration of TFC 0.055 µg CE/g, and least concentration 0.038 µg CE/g of TFC obtained in acetone extract (Table 5).

Samples	Organic Extracts	M. domestica	C. verum	T. ammi
TPC (mg GAE/g) Mean ± SD	Aqueous	72.15 ± 0.01^{a}	3.49 ± 0.01^{c}	0.197 ± 0.01^{a}
	Methanolic	66.15 ± 0.02^{c}	21.0 ± 0.05^{a}	0.845 ± 0.04^{d}
	Ethanolic	64.50 ± 0.01^{d}	7.45 ± 0.05^{b}	0.210 ± 0.03^{b}
	Acetonic	70.12 ± 0.01^{b}	3.45 ± 0.01^{d}	0.429 ± 0.01^{c}
TFC (μg CE/g) Mean ± SD	Aqueous	12.75 ± 0.02^{c}	5.255 ± 0.05^{c}	0.040 ± 0.01^{c}
	Methanolic	15.62 ± 0.05^{b}	8.56 ± 0.04^{a}	0.051 ± 0.03^{a}
	Ethanolic	13.77 ± 0.05^{a}	3.124 ± 0.03^{d}	0.055 ± 0.05^{b}
	Acetonic	12.50 ± 0.01^{d}	6.23 ± 0.00^{b}	0.038 ± 0.01^{d}
DPPH (%) Mean ± SD	Aqueous	52.25 ± 0.05^{b}	19.69 ± 0.05^{a}	82.90 ± 0.01^{c}
	Methanolic	$123.0. \pm 0.01^{a}$	109.8 ± 0.04^{d}	83.45 ± 0.05^{a}
	Ethanolic	103.11 ± 0.05^{c}	20.45 ± 0.01^{b}	80.50 ± 0.03^{b}
	Acetonic	56.31 ± 0.04^{d}	15.45 ± 0.05^{c}	79.75 ± 0.01^{c}
Reducing potential (A_{max} at 700 nm) Mean ± SD	Aqueous	0.75 ± 0.01^{b}	13.49 ± 0.05^{c}	2.78 ± 0.01^{a}
	Methanolic	0.45 ± 0.01^{c}	0.97 ± 0.05^{c}	1.18 ± 0.03^{c}
	Ethanolic	0.43 ± 0.03^{d}	0.42 ± 0.01^{d}	2.73 ± 0.01^{b}
	Acetonic	0.30 ± 0.04^{a}	10.38 ± 0.01^{b}	2.94 ± 0.04^{d}

Table 5. Antioxidant properties of organic extracts of *M. domestica*, *C. verum* and *T. ammi;* ($p < 0.05$) with 95% confidence, mean carrying different superscripted alphabets (a-d) were significantly different among means.

DPPH radical scavenging assay A methanol extract from *M. domestica* had shown the highest values of percentage inhibition 123.0% and distilled water extracts showed the lowest values of percentage inhibition 52.25%. The *C. verum* had maximum inhibition in methanol extracts 109.8% and the lowest inhibition had shown in acetone extract 15.45%. The methanol extracts exhibited the highest percentage inhibition of *T. ammi* 83.45%, while acetone extracts exhibited the lowest 79.75% (Table 5).

Saeed et al. (2017) reported that DPPH radical scavenging potential of aqueous extract of *Cinnamomum verum* had (19.52%) that is slightly different from our results.

Reducing power The reducing ability can be reveal the active antioxidant potential of any solution. The *M. domestica* can reduce from 0.30 to 0.75 in the visible range. Aqueous extracts have the highest potential 0.75, while acetone extracts have the lowest 0.30. The maximum reducing potential 13.49 of *C. verum* was obtained in aqueous extract at 700 nm while minimal potential 0.42 got through ethanol extract. *T. ammi* has a reducing power in the range of 1.18–2.94 at 700 nm. Acetone extracts had the highest reducing potential 2.94 and least potential 1.18 in methanol extracts (Table 5).

Based on a biological evaluation and comparison of C. *verum*, *T. ammi* and *M. domestica* .It is concluded that C. *verum* is more effective as compared to *T. ammi* and *M. domestica* regarding biological activities. Additionally *C. verum*, *T. ammi*, and *M. domestica* organic extracts contained significant antimicrobial and antioxidant activities that provided further opportunities for discovery in the herbal drug industry.

Discussion

The widespread increase of antibiotic resistance in microorganisms[48]and increasing demand of potent antioxidants have driven the research society to focus on the naturally produced bioactive components as potential antimicrobial and antioxidants[49]. The popularity of plant-based products has been increasing now-a-days due to their wide range of properties i.e. antioxidant, antimicrobial, and anti-inflammatory etc[50]. All these properties are accredited to the variety of phytochemicals present in plants including terpenoids, flavonoids, tannins, phenolic compounds and essential oils[51]. Due to all these properties, *Trachyspermum ammi*, *Cinnamomum verum*, and *Malus domestica*have gained fame in the therapeutic medicines[52,53].

The primary objective of the current work is to determine the antioxidant ad antibacterial potential of crude extracts from selected plants i.e. Malus domestica, Cinnamomum verum, and Trachyspermum ammi. Different standardized assays were employed i.e. well-diffusion and MIC for antibacterial activity, and DPPH assay for antioxidant activity. This study has provided comprehensive understanding about the potent biologically active compounds from crude extracts of different plants. The antibacterial potential of *M. domestica*, *C. verum* and *T. ammi* was determined through agar well diffusion method in which two different strains gram-positive strain *Staphylococcus aureus* and gram-negative strain *Salmonella typhi* were used. Some of these extracts were shown excellent antibacterial activity with clear zones of inhibition while other showed very less no zones of inhibition.

The maximum antibacterial activity was observed for the methanolic extract having 22.3 ± 0.58 mm and acetonic extract 22.3 ± 0.58 mm zone of inhibition of *Cinnamomum verum* against *Staphylococcus aureus* whereas acetonic extract showed maximum inhibition against *Salmonella typhi* with 23 ± 0.01 mm clear zone of inhibition. It was reported that the highest zone of inhibition was observed for the ethanolic extract (27.5 mm)

and the acetonic extracts (24.5 mm) against *Staphylococcus aureus* whereas, methanolic extract showed highest zone of inhibition (25 mm) against *Bacillus subtilis*[54]. In our study the value of zone of inhibition is different this might be due to the difference in environment or handling of protocol. A similar study was conducted in 2018; they had determined the antibacterial activity of *Cinnamomum verum* bark extract against broad range of microorganisms. Their results showed similar zones of inhibitions against *Salmonella typhi*(16–22 mm) hence they are the supportive evidence of our study[55]. Various research studies have confirmed the antibacterial efficacy of *Cinnamomum verum*[56,57]. Rifampicin used as positive control and DMSO as negative control. The antibacterial activity of *Malus domestica* showed highest zone of inhibition was observed against both bacterial strains *S. aureus* and *S. typhi* from methanolic extract with 18 mm clear zone of inhibition.

The highest zone of inhibition through *Trachyspermum ammi* was observed through methanolic extract 20.3 ± 0.58 mm and ethanolic extract 20 ± 0.01 mm against *Staphylococcus aureus*. For *Salmonella typhi*, the highest zone of inhibition 22.3 ± 0.58 mm was observed through ethanolic extract. Arif et al. reported that aqueous extract of *T. ammi* showed zone of inhibition against the *B. subtilis* 1 ± 0.4 cm and *N. sativa* showed antibacterial activity against the *S. aureus* 0.6 ± 0.36 cm[58]. The minimum inhibitory concentration of these plant extracts was determined which showed the excellent antibacterial potential of crude extracts from these medicinal plants. MIC ensures the potent antibacterial efficacy of bioactive components as it measures the lowest concentration of extract required to inhibit visible bacterial growth. It allows the accurate comparison of various antimicrobial agents present in medicinal plant extracts. In therapeutic applications, MIC helps to determine the exact practical concentration of antibacterial compound to halt microbial growth while reducing waste or potential toxicity.

The antioxidant potential of *M. domestica*, *C. verum* and *T. ammi*was evaluated through their TPC, TFC and DPPH assays analysis. These plants possess total phenolic and flavonoid compounds which help to reduce the cellular damage caused by reactive oxygen species. These ROS serve as a crucial factor in various chronic diseases. Different mechanisms are performed by these compounds to reduce the impact of ROS on the cellular life span i.e. scavenging free radicals, regulating antioxidant enzymes and chelating metal ions (cooper and iron) which usually catalyze the synthesis of reactive oxygen species. These compounds have hydroxyl groups which transfer their hydrogen atoms to these free oxygen atoms or free radicals, in results neutralize them[59,60].

Aqueous *M. domestica* extracts had the highest concentration 72.15 mg GAE/g, In *C. verum*, the maximum concentration of TPC was observed in methanol extract of *C. verum* 21.0 mg GAE/g. Methanol extract of *T. ammi* in displayed the highest TPC 0.845 mg GAE/g. These results showed that the total phenolic content of solution is also depending on the nature of solvent. Different extracts have different level of phenolic content and showed difference in antioxidant potential. Saeed et al. experimented seven Egyptian spices (clove, cinnamon, basil, fennel, thyme, juniper and ginger) for their antioxidant activity and reported that the phenolic content of aqueous extract of *Cinnamomum verum* has (3.38 mg GAE/g) that is slightly different from our results, as we have obtained 3.49 mg GAE/g of TPC in aqueous extract of *Cinnamomum verum*[61].

The *M. domestica* had the highest concentration of TFC in methanol extracts 15.62 μg CE/g. The organic extract of *C. verum* had maximum TFC concentration in methanol extract of *C. verum* 8.56 μg CE/g. The *T. ammi* extract in ethanol showed highest concentration of TFC 0.055 μg CE/g. Asale et al. in their study analyzed sugar, vitamin C, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of three varieties of whole *Malus domestica*fruits (peel and flesh) (Golden delicious, Fuji rose, and Granny smith)[62].

DPPH assay was performed in order to determine the electron donating ability of antioxidants present in plant extracts. These antioxidants help to neutralize the DPPH radicals through transferring electrons, which is confirmed through the color changes from purple to yellow. Hence, this assay ensures the presence of powerful antioxidants in the plant extracts. DPPH assay analysis revealed the excellent antioxidant properties of plant's crude extracts i.e. *M. domestica* 123% inhibition, *C. verum* 109.8% and *T. ammi* 83.45%. Furthermore, reducing power assays were also employed for further confirmation as this assay involved the mechanism of donating electrons to ferric ions (Fe^{3+}) and converts them into ferrous ions (Fe^{2+}). This assay interrelated with the extract's ability to respond ROS and replace them into less harmful substances[63].

The findings of the current work emphasized the therapeutic potential of biologically active compounds present in the crude extracts of plants. These plants exhibit excellent antioxidant and antibacterial properties provide evidence for future research where the specific bioactive components can be isolated and characterized from *M. domestica*, *C. verum* and *T. ammi* to get benefit in medicines, therapeutics, and preservation of food products.

Conclusion

Plants have long been the backbone of traditional healing systems around the world, as well as an important component of history and culture. The organic extracts of *C. verum*, *T. ammi*, and *M. domestica* are beneficial source of powerful antimicrobial and antioxidant compounds in potential concentration. This study verified that these plants have prominent antibacterial and antioxidant efficacy which can be ascribed to the potential phytochemicals including flavonoids, terpenoids, phenolic compounds, and tannins. They are the source of medicinally active ingredients and have multiple pharmacological effects, so it is encouraging to find its new therapeutic applications.

Data availability

The study data are present in the main text, and for further inquiries please contact the corresponding author.

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Author contributions

Conceptualization, P.M. and A.A, U.I.; Methodology, Q.M., U.I. and R.J.; Software, U.I. A.S.; Validation, Q.M., P.M. and A.A.; Formal Analysis, U.I. and P.M.; Investigation, Q.M. U.I., A.S.; Resources, P.M.; Data Curation, A.S., U.I.; Writing – Original Draft Preparation, U.I.; Writing – Review & Editing, P.M. and A.A. Q.M.; Visualization, R.J., A.S.; Supervision, P.M. and A.A.; Project Administration, P.M. A.A.; Funding Acquisition, P.M. and U.I.

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Declarations

Competing interests

The authors declare no competing interests.

Consent for publication

This research work is original, has not been previously published, and is not under consideration for publication elsewhere. Therefore, all authors give their consent for this publication.

Ethics approval and consent to participate

No Ethical concern was raised during this study as no animal or human was used for this study.

Research involving plants

The plant used in this study complied with national and international guidelines.

Additional information

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