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Short Communication
Biochemical Composition, Antioxidant and Antibacterial Activities of Commonly Used Culinary Indian Spices

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Abstract

Background: Antibiotic resistance become global concern due to over use and getting drug resistance in bacteria drawn attention for best candidate from natural resources like spices which were using since, ancient days in culinary and also in traditional medicine like Ayurveda for development of new antimicrobial compounds. **Methodology:** Methanolic extracts of 20 routinely consumed spices in Indian culinary were evaluated for their total phenolics, flavonoids, terpenoids and alkaloids with antioxidant and antibacterial potential against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*). **Results:** Results revealed that higher levels of total phenolics were noticed in clove (175±21 mg GAE g^{-1}) followed by star anise, bay leaf and caper. Terpenoid rich caper (639±19 mg LE g^{-1}) exhibited elevated levels of antioxidant potential (642±6 mg TE g^{-1}) as compared to other spices. Turmeric exhibited highest amount of flavonoids (31.4±2.34 mg RE g^{-1}) followed by clove and black pepper. Mustard showed higher alkaloid content (1.6±0.08 mg AE g^{-1}) followed by chilli and black pepper. The Gram-positive and Gram-negative bacteria were exhibited an increased growth inhibition (antibacterial and bactericidal activities) at lower concentration of garlic (2.5 µg GAE $disc^{-1}$), cinnamon (4.5 µg GAE $disc^{-1}$) and tamarind extracts (6 µg GAE $disc^{-1}$) as compared to the standard antibiotic and streptomycin (20 µg $disc^{-1}$). **Conclusion:** The results concluded that the phenolics and terpenoid rich spices exhibited elevated antioxidant, antibacterial and bactericidal activities. Indian spices could also be used as potential antimicrobials that develop the promising leads to the pharmaceutical industry.

Key words: Indian spices, bioactive compounds, antioxidants, antibacterial activity, bactericidal activity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotic resistance in bacteria has become a major global concern and primarily due to antibiotic overuse. According to the WHO 2014 report on global surveillance of antimicrobial resistance, the antibiotic resistance is no longer a prediction for the future and is the happening right now across the world, putting the globe at risk and the ability to treat common infections in the community and hospitals[1]. Resistance to antibiotics can occur in bacteria through several means like by the modification of target sites, molecular bypass, active efflux and altering the drug etc[2]. A major class of antibiotics had discovered between 1930 and 1960. Increasing use of antibiotics leads to an increase in antibiotic resistance among bacteria occurred but not a parallel increase in new agents with a significant improvement in the spectrum of activity. From 1968 to date and only five antibiotics were discovered[3]. Teixobactin was the antibiotic discovered in 2015[4]. Hence, this alarms to discover safe and potential antibiotics that may overcome the risk of side effects, cost effectiveness and for which bacteria doesn't get resistant.

The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity towards host cells are considered as best suitable candidates for developing new antimicrobial drugs. Plants turn out to be the useful source for this problem due to their effective bioactive compounds[5]. Nevertheless, the route of this therapy was from the ancient tradition practice like Ayurveda, Siddha and Unani[5,6]. However, the compounds involved in antimicrobial activity and their mechanism of action has not been fully understood. In an effort to expand the spectrum of antimicrobial compounds from natural resources, Indian spices were chosen for this study. From ancient days, Indian spices were playing a major role not only in culinary but also in traditional medicine like Ayurveda, Siddha and Unani. Various activities of spices include antibacterial[7], antifungal[8], anticancer[9], antidiabetic[10], antioxidant[11], food preservative[12] and cholesterol-lowering effect[13] and also influence on body metabolism[14]. According to the previous study, major attention dragged over medicinal plants against bacteria while, focusing less on Indian spices though performing greater potential biological activities[5]. No studies have been reported on biochemical evaluation of all major secondary metabolites particularly terpenoids and alkaloids etc. and their antioxidant and antimicrobial activities in large number of spices to identify an effective spice which shows potential antibacterial and bactericidal activity with their enriched bioactive compounds. Hence, this study has 20 routinely consuming spices in Indian culinary for evaluation of bioactive compounds (Total phenolics, terpenoids and alkaloids) antioxidant activities and their antibacterial and bactericidal activity against two Gram-positive bacteria (*S. aureus* and *B. subtilis*) and two Gram-negative bacteria (*E. coli* and *K. pneumonia*).

MATERIALS AND METHODS

Materials: All routinely used chemicals and reagents were purchased from Merck Specialties Pvt. Ltd, Mumbai, India and Thermo Fisher Scientific (India) Pvt. Ltd, Mumbai, India. Rutin hydrate, trolox ((±)-6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), Linalool, ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), atropine sulfate, INT (Iodonitrotetrazolium chloride), gallic acid were purchased from the Sigma-Aldrich, St. Louis, MO, USA. All the antibiotics and other useful chemicals were purchased from Himedia laboratories Pvt. Ltd, Mumbai, India. Bacterial cultures, *Klebsiella pneumonia* (MTCC-432), *Escherichia coli* (MTCC-443), *Staphylococcus aureus* (MTCC-87) and *Bacillus subtilis* (MTCC-10619) were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India.

Plant material collection: All the spices were procured from the local market, identified and deposited in the Herbarium, Department of Botany, Yogi Vemana University, Kadapa, India with voucher numbers (Table 1). They were made to a fine powder and sewed using 0.2 mm sieve and stored until further use in a sealed cover at room temperature. Onion and garlic were used without drying.

Preparation of spices extractions: Extractions were performed under cold conditions taking 2 g of spices powder dissolved in 80% methanol with the sample to solvent ratio 1:10 (2 g in 20 mL solvent) followed by incubation in a shaking incubator for overnight at 10°C. The extracts were centrifuged at 4000 rpm for 5 min, the supernatants were collected and pellets were reused for extraction. Fresh solvents were added to the pellets remained and kept on shaking incubator for 3 h for extraction. The procedure was repeated twice in case of all the spices so that all the bioactive compounds present in the powder will be diffused completely into the extraction solvent. Collected supernatants were pooled, concentrated in vacuum under reduced pressure by using rotary evaporator (Heidolph Rotary Evaporator, Germany). Concentrated extracts were obtained, aliquoted and stored at -80°C until use.

Total phenolics assay: Total phenolics were estimated as described by Singleton *et al.*[15] with slight modification.

Approximately, 140 µL of the extract or standard gallic acid was added to 600 µL of the 0.2 M Folin-Ciocalteu reagent. After 5 min, 460 µL of 7.5% (w/v) sodium carbonate was added. The reaction mixture was incubated in dark at 45°C for 30 min and followed by 1 h incubation at room temperature. Absorbance was measured at 765 nm against solvent blank. Results were expressed as mg gallic acid equivalents per gram of spice (mg GAE g^{-1}).

Alkaloids assay: Alkaloids were determined following Novelli *et al.*[16] with slight modification. To 1.0 mL of extract in a separating funnel, 5 mL of bromocresol green (BCG), 5 mL of 0.1 M phosphate buffer (pH 4.7) were added and shaken vigorously. The BCG forms complex with alkaloids present in the extract. Later added 5 mL of chloroform, this entire solution turns to yellow. Colored complex formed was separated carefully and read absorbance at 470 nm against blank having chloroform and BCG without any extract. Atropine was taken as standard and the result was expressed as mg atropine equivalents per gram of spice (mg AE g^{-1}).

Terpenoids assay: Terpenoids were estimated by using the protocol developed by Ghorai *et al.*[17]. Two hundred microliters extract was added to 1.5 mL of chloroform after 3 min 100 µL of the conc. H_2SO_4 was added. The heat generated at this step was reduced by keeping the entire setup on ice for less than 15 min and followed by incubation for 90-120 min in the dark. Linalool was used as standard and incubated for 5 min and observed red precipitate at the bottom of tube. The supernatant was decanted carefully without disturbing the pellet. To the pellet, 1.5 mL of 80% methanol was added and read absorbance at 538 nm. Results were expressed as mg of linalool equivalents per gram (mg LE g^{-1}) of spice.

Flavonoids assay: The aluminum chloride colorimetric method was used for flavonoid determination adopted from Pourmorad *et al.*[18]. To 25 µL of extract, 75 µL of 95% alcohol was added, followed by 5 µL 10% aluminum chloride and 1M potassium acetate. Final volume was made upto 260 µL with distilled water. Incubated at room temperature for 40 min and read absorbance of the reaction mixture at 415 nm. Results were expressed as mg of rutin equivalents per gram (mg RE g^{-1}) of spice.

Antioxidant activity (ABTS) assay: The ABTS free radical scavenging assay was performed by following the protocol described by Re *et al.*[19]. Before 16 h of the experiment, mother solution of ABTS was prepared by mixing equal volumes of 8 mM ABTS and 3 mM potassium persulfate and allowed to react in dark condition for the generation of ABTS free radicals. Before starting the experiment, working solution of ABTS was prepared by adding 1 mL of the mother solution to the 29 mL of the 0.2 M phosphate buffer (pH 7.4). To 10 µL of the spice extract, 290 µL ABTS working solution was added and incubated at room temperature for 30 min. Then, discoloration of ABTS solution due to the antioxidant nature of the extract was measured at 734 nm. Trolox was taken as standard antioxidant and results were expressed as mg of trolox equivalents per gm (mg TE g^{-1}) of the spice.

Reducing power assay: Reducing power assay was performed by following method of Ferreira *et al.*[20]. Aliquots of 10, 20, 30 and 40 µL of extract were taken in a 5 mL test tube and volume was made up to 400 µL with distilled water. To that 500 µL of 0.2 M phosphate buffer (pH 6.6) and 500 µL of 1% potassium ferricyanide were added and kept in the water bath at 50°C for 20 min. Then, tube was removed and added 10% trichloroacetic acid to stop the reaction and centrifuged at 3500 rpm for 10 min. The upper layer (0.5 mL) was collected and added 0.5 mL of distilled water and 100 µL of 0.1% ferric chloride. Absorbance was read at 700 nm. Ascorbic acid was used as a standard and results were expressed as EC_{50} where EC_{50} stands for effective concentration of spice extract in µg GAE g^{-1} at which absorbance was 0.5.

Determination of antimicrobial activity of Indian spices: Antibacterial activity was performed by disc diffusion method developed by Bauer *et al.*[21]. Extracts of the spices were evaluated against two Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) and two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*).

Preparation of bacterial inoculum: Loop full of 24 h culture of respective bacteria was taken and dropped in 5 mL of nutrient broth contained in a tube and incubated in a shaking incubator at an optimum temperature until the culture reached 0.5 McFarland units which have bacterial concentration of $1.5x10^8$ CFU.

Determination of antimicrobial activity: Bacterial culture was spread over the Muller-Hinton agar plate with a sterile cotton swab. At four corners of the seeded petri plate, Whatman No.1 filter paper discs of size (6 mm) were placed. Twenty microliters of methanol extract(s) of Indian spices were dropped over discs. The entire set-up was incubated at 37°C

for 24 h, the zone of inhibition (ZOI) was measured after incubation and the results were expressed in mean of ZOI in mm. Streptomycin was used as the standard antibiotic.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): The MIC was performed as per the American Society of Microbiology (ASM) manual according to CLSI guidelines and by Eloff[22] methodology with slight modification. The MIC and MBC of test samples were determined as follows. To 1 mL of the nutrient broth in the test tubes and added different concentrations of spice extract of interest. Log phase culture of interested bacteria were taken and checked the absorbance of the culture in the spectrophotometer (0.5-0.8 OD values gives 1.5×10^8 CFU mL^{-1}). Culture was diluted 20 times and added 100 µL of the diluted culture directly to the tubes and incubated for 16 h. Then, 40 µL of 0.2 mg mL^{-1} INT was added to the tubes and incubated at 37°C for 30 min. After incubation colour change (colourless or yellow to pink) was noticed in the tubes indicating bacterial growth and no colour change in the tubes indicating absence of bacterial growth and that concentration was considered as MIC concentration.

For the determination of the MBC, a portion (10 µL) of the above MIC experimental solution from the tube at which no colour change was observed was taken and spread over the sterile Muller-Hington agar plate, incubated for 24 h, observed the plate in which no bacterial colonies were noticed and it was considered as MBC concentration. The MIC and MBC were expressed as µg of GAE in extract per tube.

Statistical analysis: Each value in the data presented in the table represents the arithmetic mean or Mean±SE of five independent determinations and unless otherwise stated. Data were represented as Means±Standard Error (SE). Statistical significance was determined by one-way ANOVA.

RESULTS

Enriched bioactive compounds of spices showed higher antioxidant properties: Twenty routinely consumed culinary spices were grinded to fine powders and extracted in 80% methanol. The extract yield was variable from one spice to other (tamarind-13% to onion-5%). The individual extracts were tested for their total phenolics, flavonoids, terpenoids, alkaloids and antioxidant potential. Great variation was observed in the contents of above bioactive compounds in all tested spices (Table 1). Higher levels of phenolics in order were noticed in clove (175±21 mg GAE g^{-1}) followed by star anise (159±8.9 mg GAE g^{-1}), bay leaf (138±17 mg GAE g^{-1}) and caper (105±11 mg GAE g^{-1}). Terpenoids in spices also varied greatly with 639±19 mg LE g^{-1} caper stood first followed by cinnamon (399±17 mg LE g^{-1}) and bay leaf (363±14 mg LE g^{-1}). Though garlic was poor in phenolics but showed higher terpenoid content with 311±15 mg LE g^{-1}. Lower levels of alkaloids were noticed in all the tested spices (Table 1). Among 20 spices, mustard showed higher alkaloid content (1.6±0.08 mg AE g^{-1}) followed by chilli and black pepper. Turmeric exhibited highest amount of flavonoids (31.4±2.34 mg RE g^{-1}) followed by clove and black pepper (16±0.4 mg RE g^{-1}). Caper, clove, cinnamon and bay leaf extracts showed highest antioxidant potential by ABTS activity with 642±6, 512±13, 256±23 and 170±12 mg TE g^{-1}, respectively. This study explained a strong correlation between phenolics and ABTS activities with an R^2 value of 0.9331 (Fig. 1). Cinnamon and star anise showed potential reducing power activities even at lower effective concentration (EC_{50}) of 2.67 and 3.19 µg, respectively (Table 1).

Spices extracts suppresses bacterial growth and exhibits bactericidal activity: Antibacterial activity zone of inhibition (ZOI) in mm Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) were evaluated in routinely used Indian spices and results depicted in Table 2. All the spices used exhibited a great variation in antibacterial activity. This study explained that both Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*E. coli* and *K. pneumonia*) showed more susceptibility against ajowan, garlic, cinnamon and tamarind

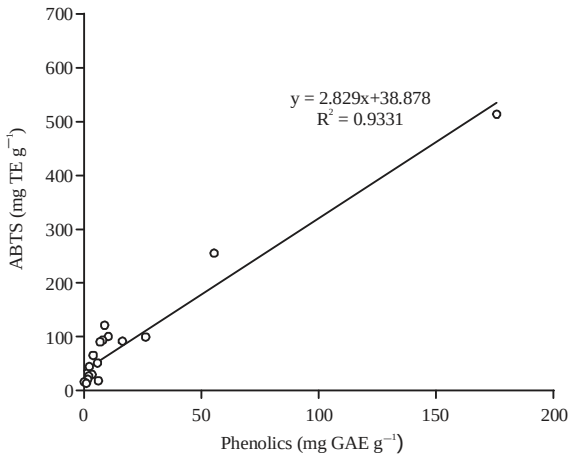

Fig. 1: Relationship between the antioxidant capacity (ABTS free radical scavenging activity) and total phenolic content of methanolic extracts of Indian spices

Table 1: Levels of phenolics, flavonoids, terpenoids, alkaloids, ABTS radical scavenging activity and reducing potential of the methanolic extracts of Indian spice (s)

Plant name	Scientific name	Part used	Voucher No.	Phenolics (mg GAE g^{-1})	Flavonoids (mg RE g^{-1})	Terpenoids (mg LE g^{-1})	Alkaloids (mg AE g^{-1})	ABTS (mg TE g^{-1})	Reducing potential (EC_{50} in µg GAE g^{-1})
Ajowan	*Trachyspermum ammi* S	Seed	YVUH4722	25.80±3.2	4.4±0.1	111.0±5	0.605±0.045	100.0±9.7	17.12±1.20
Bay leaf	*Laurus nobilis* L	Leaf	YVUH 4723	138.00±17	3.8±0.1	363.0±14	0.310±0.036	170.0±12.0	54.92±2.60
Black pepper	*Piper nigrum* L	Fruit	YVUH 4724	5.50±0.7	16.2±0.5	80.0±3.7	0.740±0.034	52.1±7.6	11.88±0.80
Caper	*Capparis spinose* L	Bud	YVUH 4725	105.00±11	2.8±0.02	639.0±19	0.100±0.012	642.0±6.1	22.68±1.30
Cardamom	*Elettria cardamom* L	Seed	YVUH 4727	2.30±0.29	0.9±0.1	57.0±3.2	0.140±0.008	45.2±2.2	6.26±0.21
Chilli pepper	*Capsicum frutescens* L	Fruit	YVUH 4728	6.50±0.82	6.1±0.3	85.0±6.2	1.000±0.006	90.8±8.0	18.1±0.94
Cinnamon	*Cinnamomum zeylancium* B	Bark	YVUH 4729	55.00±6.8	3.3±0.1	399.0±17	0.070±0.003	256.0±23.0	2.67±0.03
Clove	*Syzygium aromaticum* L	Bud	YVUH 4731	175.00±21	16.0±0.4	108.0±8	0.320±0.012	512.0±13.0	15.40±0.90
Coriandrum	*Coriandrum sativum*	Seed	YVUH 4732	1.50±0.19	1.5±0.05	51.0±4.1	0.330±0.0087	22.6±1.7	6.16±0.70
Cumin seeds	*Cuminum cyminum* L	Seed	YVUH 4733	10.20±1.2	11.0±0.4	51.7±3	0.320±0.0067	102.0±5.8	15.45±1.40
Curry leaf	*Murraya koenigii* L	Leaf	YVUH 4734	6.00±0.75	2.5±0.07	47.8±4	0.440±0.0085	20.4±0.8	30.33±1.70
Garlic	*Allium sativum* L	Bulb	YVUH 4735	0.25±0.03	0.3±0.01	311.0±15	0.250±0.01	19.7±1.3	13.70±0.68
Ginger	*Zingiber officinale* R	Rhizome	YVUH 4736	1.10±0.14	0.8±0.01	38.0±2.2	0.260±0.05	14.9±1.5	12.59±0.90
Mustard	*Brassica juncea* L	Seed	YVUH 4737	3.30±0.41	1.2±0.09	71.2±8	1.600±0.0876	32.3±3.4	17.52±1.30
Onion	*Allium cepa* L	Bulb	YVUH 4738	0.16±0.02	0.4±0.01	28.2±2	0.070±0.0006	14.4±0.5	25.23±2.90
Star anise	*Illicium verum* H	Fruit	YVUH 4739	159.00±8.9	2.8±0.02	176.0±9	0.300±0.0063	92.3±9.7	3.19±0.50
Tail pepper	*Piper cubeba* L	Fruit	YVUH 4740	7.45±0.93	7.8±0.2	56.0±3.6	0.400±0.0056	95.0±2.4	4.85±0.10
Tamarind	*Tamarindus indica* L	Fruit	YVUH 4741	1.90±0.03	13.0±0.1	160.0±4	0.140±0.02	80.0±2.8	7.16±0.70
Trigonella	*Trigonellafoenumgracum* L	Seed	YVUH 4742	4.20±0.25	6.3±0.1	76.9±4	0.590±0.0072	65.0±2.9	22.36±1.50
Turmeric	*Curcuma longa* L	Rhizome	YVUH 4743	8.30±0.76	31.4±2.3	102.0±8	1.200±0.08	121.0±9.5	13.52±0.80

GAE: Gallic acid equivalents, RE: Rutin equivalents, LE: Linalool equivalents, TE: Trolox equivalents, AE: Atropine equivalents and EC_{50}: Half maximal effective concentration, EC_{50} of ascorbic acid-7.2 µg and each value presented in table represents the Mean±SE of five independent determinations

Table 2: Antibacterial activity zone of inhibition (ZOI) Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the methanolic extracts of Indian spice(s) against Gram-positive and Gram-negative bacteria

	Mean of zone of inhibition (ZOI) in mm, MIC and MBC (μg mL^{-1})											
	Gram-negative						Gram-positive					
	Escherichia coli			*Klebsiella pneumonia*			*Staphylococcus aureus*			*Bacillus subtilis*		
Spice used	ZOI	MIC	MBC	ZOI	MIC	MBC	ZOI	MIC	MBC	ZOI	MIC	MBC
Ajowan	32	25	50	19	50	75	23	25	25	35	25	25
Bay leaf	17	50	100	15	100	150	14	150	150	22	50	75
Black pepper	19	75	100	16	100	150	17	100	150	14	150	ND
Caper	25	50	75	ND	ND	ND	17	150	175	18	75	125
Cardamom	15	100	150	19	75	125	18	75	125	21	75	125
Chilli pepper	18	100	125	17	100	150	19	100	125	22	50	100
Cinnamon	25	25	25	27	25	25	21	25	25	19	25	50
Clove	17	25	50	19	25	50	16	100	150	23	50	75
Coriandrum	15	100	150	16	150	ND	13	200	ND	13	150	ND
Cumin seeds	15	75	125	16	100	150	15	100	150	16	150	ND
Curry leaf	15	100	150	17	100	150	15	150	200	19	100	150
Garlic	25	25	25	19	25	25	18	25	25	19	25	50
Ginger	11	125	ND	ND	ND	ND	11	ND	ND	11	150	ND
Mustard	ND	ND	ND	15	100	150	12	150	ND	16	125	175
Onion	15	150	ND	15	150	175	13	200	ND	ND	ND	ND
Star anise	19	25	50	20	25	50	19	50	75	19	75	100
Tail pepper	25	50	75	19	75	125	12	150	ND	28	50	75
Tamarind	27	25	25	29	25	25	23	25	25	30	25	25
Trigonella	ND	ND	ND	ND	ND	ND	ND	ND	ND	14	150	200
Turmeric	17	50	75	15	100	125	18	75	100	15	100	150
Streptomycin	23	25	25	22	20	20	23	20	20	22	20	20

ND: Not detected and each value presented in table represents the arithmetic mean of five independent determinations

with higher ZOI at lower concentrations of MIC and MBC (<25 μg GAE of extract per well) however, lesser susceptibility was detected with ginger and mustard. Trigonella did not show any antibacterial activity. Some of the spice(s) extracts (Garlic 2.5 μg GAE disc^{-1}, cinnamon 4.5 μg GAE disc^{-1}, tamarind 6 μg GAE disc^{-1} and ajowan 25 μg GAE disc^{-1} etc.) showed more antibacterial and bactericidal activity at lower concentrations than the standard antibiotic and streptomycin (20 μg disc^{-1}).

DISCUSSION

There are two major reasons for the researchers to turn their interest towards the plant remedies in treating many diseases. They are short life expectancy and failure of the newly synthesized drug regimens. For centuries, spices in Indian culinary have made a significant contribution both in the health care system, particularly in Ayurveda, Siddha and Homeopathy and also contributed a major amount for the treatment of key disorders of the body. In this respect, the spices were chosen for this study because of their very good potential medicinal properties. In support, this study identified the enriched secondary metabolites in all the tested spices, namely phenolics, terpenoids, alkaloids and flavonoids[23]. Phenolics plays a major role of protection in the plant systems against various microorganisms[12]. In the present study, clove, bay leaf, caper, star anise and cinnamon showed higher levels of total phenolics (Table 1). This study results were in consistent with other reports of chilli pepper[24], clove and black pepper[25], cinnamon, cumin and curry powder[26], tamarind[27], star anise and cinnamon[28], bay leaf[29] and garlic[30]. Alkaloids are seemingly very less in all tested spices except mustard. Flavonoids afford colour to the plants along with anthocyanins and majorly functions in photosynthesizing cells[31]. Flavonoids, the major group of phenolics attribute antimicrobial nature to the extracts[32]. Apart from the phenolics, this study also reported terpenoids are also a chief group of secondary metabolites present in spices extracts involving antimicrobial activity (Table 1, 2). Garlic showed very fewer phenolics but were good at terpenoids showed high antimicrobial activity. Indian spices shown elevated levels of antioxidant activities and have a strong correlation with phenolics and ABTS.

Bioactive compounds enriched Indian spices showed good antimicrobial activity on both Gram-positive and Gram-negative bacteria. Previous studies demonstrated that the spices were more active against bacteria[12] and reported that there is a strong correlation between the antioxidant activity and microbial activity. Clove, caper and bay leaf

showed higher phenolics with elevated antioxidant potential which further responsible for potential inhibition of bacterial growth even at lower MIC and MBC (Table 1, 2). Tamarind, garlic, cinnamon and ajowan displayed higher antibacterial and bactericidal activity even at lower concentrations of extracts. The results of this study are in line with previous reports and revealed higher phenolics or terpenoids in the spice(s) that are responsible for their antimicrobial nature viz., cinnamaldehyde of cinnamon inhibits bacteria by affecting the membrane integrity, thymol of ajowan and allicin of garlic suppresses the growth by inducing the cellular leakage[33]. Tamarind offered a good antibacterial and bactericidal activities against all tested bacteria with enriched flavonoids and terpenoids[34,35]. Ajowan containing thymol as the major bioactive compound exhibited good antimicrobial activity against all the tested bacteria. Apart from thymol, γ-terpinene, o-cymene and carvacrol are the other major compounds in ajowan responsible for good antimicrobial and antifungal activities[36].

CONCLUSION

This study concluded that higher levels of phenolics, terpenoids and flavonoids in routinely used culinary Indian spices exhibit elevated antioxidant potential and antimicrobial activity which is parallel with standard antibiotic streptomycin. These results indicate that the Indian spices might serve as a major source of attractive candidates for the development of antimicrobial drugs. Further investigations are in progress on the phytochemical (ingradient) composition, isolation and identification of antimicrobial activity shown bioactive compound(s).

SIGNIFICANT STATEMENTS

- Methanolic extracts of 20 routinely consumed spices in Indian culinary were evaluated for their total phenolics, flavonoids, terpenoids and alkaloids with antioxidant potential and antibacterial activity against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*)
- Among the 20 spices tested for phytochemical evaluation, caper, clove, cinnamon, bay leaf and star anise were exhibited an elevated ABTS activity (antioxidant potential) with their enriched bioactive compounds (total phenolics, terpenoids and flavonoids) as compared to other spices. Also, observed a strong correlation between total phenolics and ABTS activities with an R^2 value of 0.9331
- Cinnamon and star anise showed potential reducing power activities even at lower effective concentration (EC_{50}) of 2.67 and 3.19 μg GAE g^{-1}, respectively
- Both Gram-positive and Gram-negative bacteria were exhibited an increased growth inhibition (antibacterial and bactericidal activity) at lower concentration of garlic (2.5 μg GAE $disc^{-1}$) cinnamon (4.5 μg GAE $disc^{-1}$) and tamarind extracts (6 μg GAE $disc^{-1}$) as compared to other spice(s) extracts and standard antibiotic, streptomycin (20 μg $disc^{-1}$), respectively
- Terpenoid rich spices exhibited high antibacterial activity

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