Biological activities of *Allium sativum* and *Zingiber officinale* extracts on clinically important bacterial pathogens, their phytochemical and FT-IR spectroscopic analysis

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Abstract: The spread of bacterial infectious diseases is a major public threat. Herbs and spices have offered an excellent, important and useful source of antimicrobial agents against many pathological infections. In the current study, the antimicrobial potency of fresh, naturally and commercial dried *Allium sativum* and *Zingiber officinale* extracts had been investigated against seven local clinical bacterial isolates such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Streptococcus pyogenes, Staphylococcus epidermidis,* and *Serratia marcesnces* by the agar disc diffusion method. All tested pathogens except P. aeruginosa and E. coli were most susceptible to ethanolic and methanolic extracts of *A. sativum*. Similarly, chloroform and diethyl ether extracts of *Z. officinale* showed a greater zone of inhibition of tested pathogens except for *P. aeruginosa* and *E. coli*. We found that all extracts of *A. sativum* and *Z. officinale* have a strong antibacterial effect compared to recommended standard antibiotics through activity index. All results were evaluated statistically and a significant difference was recorded at $P< 0.05$. Antioxidant activity of extracts showed that 10 out of 13 extracts have high scavenging potential. Thin layer chromatography profiling of all extracts of *A. sativum* and *Z. officinale* proposed the presence of various phytochemicals such as tannins, phenols, alkaloids, steroids and saponins. Retention factor of diverse phytochemicals provides a valuable clue regarding their polarity and the selection of solvents for separation of phytochemicals. Significant inhibition of *S. aureus* was also observed through TLC-Bioautography. FT-IR Spectrometry was also performed to characterize both natural and commercial extracts of *A. sativum* and *Z. officinale* to evaluate bioactive compounds. These findings provide new insights to use *A. sativum* and *Z. officinale* as potential plant sources for controlling pathogenic bacteria and potentially considered as cost-effective in the management of diseases and to the threat of drug resistance phenomenon.

Keywords: *Allium sativum, Zingiber officinale,* TLC-bioautography, Antioxidant activity, phytochemical screening, Antibacterial activity, FTIR spectrometry.

INTRODUCTION

The capability of any microbe to cause disease is known as pathogenicity. Several diseases such as Pneumonia can be caused by *Streptococcus*, *Pseudomonas*, and food-borne illnesses, which can be caused by bacteria such as *Shigella* (Chanachai *et al*., 2008). Bacterial pathogens have the capability to cause various diseases or infections in human beings such as urinary tract, respiratory tract, eye, wound, blood, and food poisoning. Pathogenic bacteria are causative agents of various infections such Stomach pain, chill fever, diarrhea, headache, nausea, fever, pain in muscle followed by vomiting, abdominal pain, cramps and diarrhea containing blood and mucus (Esteban *et al*., 2008). Different ways are used to control or diminish the human diseases that caused by bacterial pathogens. Bacterial infections can be treated using various types of antibiotics. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains. It is essential to investigate newer drugs from natural resources with lesser resistance and the use of plants as a source of remedies for the treatment of many diseases dates back to history. The natural products have acknowledged increased attention from all over the world due to their health claims (Dobrowlski *et al*., 1991). The antimicrobial activity of various plant extracts has been reported in different parts of the world by a very large number of researchers (Kumar *et al*., 2006). Plant-based antimicrobials has been characterized as a vast unexploited source and 30% or more of the recent pharmacological drugs are direct or indirect consequent of plants and their extracts dominate in homeopathic medicines (Murugesan *et al*., 2011). *A. sativum* is therapeutically effective because of its oil and water soluble organo-sulfur compounds, thiosulfates is primarily responsible for its antibiotic activity as described by reported data that the antimicrobial capability will considerably be lost if thiosulfates (e.g., allicin) are removed from the extract (Hughes and

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Lawson, 1991). *A. sativum* has antifungal and antibacterial activity because of the presence of numerous phenolic compounds and powerful sulfur (Reuter *et al*., 1996). It is reported that allicin exhibits its antimicrobial activity mainly by direct and total inhibition of RNA synthesis, signifying that RNA is the primary target of allicin action, although, DNA and protein syntheses are also partially inhibited (Feldberg *et al*., 1988). The structural differences of the bacterial strains may also play a role in the bacterial susceptibility to *A. sativum* constituents (Tynecka and Gos, 1975). *A. sativum* exhibit a broad antibacterial activity against both Gram negative and Gram positive bacteria including species of *Klebsiella*, *Escherichia*, *Salmonella*, *Clostridium*, *Staphylococcus*, *Streptococcus*, *Proteus*, *Bacillus* and on diarrheagenic organisms (Srinivasan *et al*., 2009).

Pathogens are a major threat to cause serious infections. The natural products are found to be more efficient with minimum side effects as compared to commercial antibiotics so they have used an alternated remedy for various infectious diseases (Tepe *et al*., 2004). Historically, *A. sativum* and *Z. officinale* has been used for centuries throughout the world by a range of societies to contest infectious disease such as heart diseases, cancer, malaria, asthma, candidiasis, colds, diabetes, and also used to raise immunity (Fukao *et al*., 2007). Sahebkar, (2011) demonstrated the potential efficacy of *Z. officinale* as a natural supplement for nonalcoholic fatty liver disease. Besides that different type of toxicity by some pathogenic microbial strains can be detoxified by *A. sativum* (Singh *et al*., 2008).

In the present study, we have evaluated the antibacterial, antioxidant, phytochemical screenings, and metal chelating effect of *A. sativum* and *Z. officinale* extracts against clinically isolated pathogenic bacterial strains. Current research hypothesized that a possible significant effect of *A. sativum* and *Z. officinale* could result in the reduction of antibiotics use. Another object of present investigation was to evaluate the effect of stabilizers on the antimicrobial activity of the commercially available powder forms of *A. sativum* and *Z. officinale*.

MATERIALS AND METHODS

Ethics statement

All animal experimental procedures were conducted in accordance with local and international regulations. The international regulation referred to is the Wet op de dierproeven (Article 9) of Dutch Law.

Plant and other materials

Fresh bulbs of Garlic (*A. sativum)* was harvested in late spring from local fields (fields of the first author of this paper) of Muzaffarabad. Pakistan. The collection was performed by pulling plants out of the soil and transferring them into sealable plastic bags. That's why no specific permissions were required for these fields. It is confirmed that field studies did not involve endangered or protected species. The natural rhizome of Ginger (*Z. officinale)* was purchased from local market of Muzaffarabad and identified by an ethanol-botanist from the Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. Commercially available powder (Shan Garlic Powder and Shan Ginger Powder) were purchased for the preparation of commercially dried extracts.

Extraction of plant materials

Fresh *A. sativum* bulbs and *Z. officinale* rhizome (200 g of each) were peeled out and pulverized aseptically using pestle and mortar. Fresh extracts were prepared as 50 g crushed pieces of *A. sativum* (F.G) and *Z. officinale* (F.Z) were soaked separately in 200 ml of each organic solvent with increasing order of polarity (diethyl ether, chloroform, ethanol, and methanol) for 15-20 days. Each extract was concentrated on a rotary evaporator at 40°C dryness and used as 100% concentration. The concentrates were collected and stored at room temperature for further processing. Similarly, naturally dried (N.D) extracts were prepared as: 50 g of crushed fresh *A. sativum* (N.D.G) bulbs and *Z. officinale* (N.D.Z) rhizome were shade-dried for one week and soaked separately in 200 ml of each organic solvent for 15-20 days while for the preparation of commercially dried extracts simply 50 g of Shan commercially dried (C.D) powder of *A. sativum* (C.D.G) and *Z. officinale* (C.D.Z) were soaked into different organic solvents by using same method as mentioned above.

Physical properties

Physical properties such as color, odor and consistency of each extract of both *A. sativum* and *Z. officinale* in different organic solvents i.e. aqueous, diethyl ether, chloroform, ethanol, and methanol were recorded after 25 days of extraction.

$ABTS^{\cdot+}$ decolorization assay

$ABTS^{\cdot+}$ scavenging activity was analyzed to evaluate the antioxidant potential of *A. sativum* and *Z. officinale* extracts according to the described method of Re *et al*. (Re *et al*., 1999). The $ABTS^{+}$ stock solution was prepared by reacting potassium persulphate (2.45 mM) and $ABTS^{+}$ (7 mM), then allowing the mixture to stand for at least 16 h to generate $ABTS^{+}$ free radicals. On the other hand, the running solution was organized by diluting the stock solution with various solvents and their absorbance was recorded at 734 nm ($Ao_{Control}$). For tests, 1 ml of $ABTS^{+}$ running solution was merged with 10µl extracts of different solvents (0-100 µg/ml). The absorbance of test samples (Ai_{Sample}) was also observed at 734 nm accurately 10 min after the reaction mixture was ready. In both attempts, ascorbic acid was used as positive control. The percentage radical scavenging activity (% RSC) was

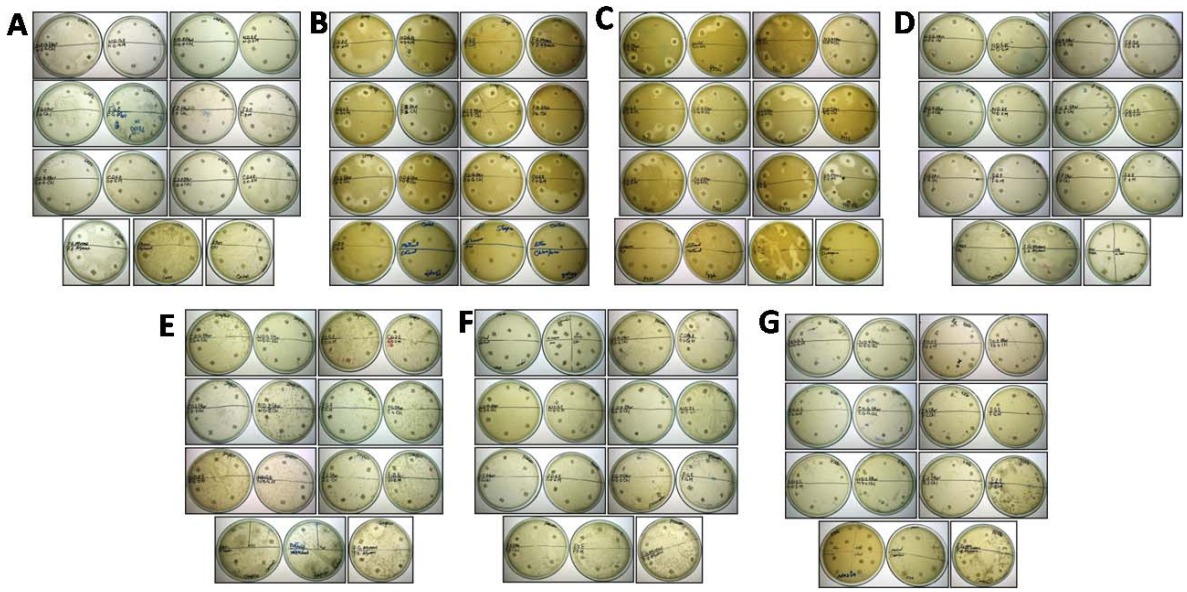

Fig. 1: Antibacterial activity of *A. sativum* and *Z. officinale* against bacterial pathogens. (A) *S. aureus,* (B) A *S. pyogenes,* (C) *S. marscenes,* (D) *E. coli,* (E) *S. epidermidis,* (F) *P. auregonosa,* (G) *K. pneumonae.*

Table 1: Physical properties of *A. sativum* and *Z. officinale* extracts

Solvent	Conc. of solvent (ml)	Quantity. Used for extracts (g)	*A. sativum*			*Z. officinale*		
			F.G	N.D.G	C.D.G	F.Z	N.D.Z	C.D.Z
Diethyl ether	200	5	CL	T	CL	LY	LL	L
Chloroform	200	5	LL	CL	CL	LL	GY	Y
Ethanol	200	5	W	CL	CL	W	G	LG
Methanol	200	5	DL	CL	LY	PY	G	DG
Aqueous	200	5	W	CL	CL	LY	G	G

F.G= fresh *A. sativum*; N.D.G= naturally dried *A. sativum*: C.D.G = commercially dried *A. sativum*; F.Z=fresh *Z. officinale*; N.D.Z=naturally dried *Z. officinale*; C.D.Z= commercially dried *Z. officinale*; CL, Colorless; T, Transparent; LL, Light lemon; DL, Dark lemon; LY, Light yellow; PY, Pale yellow; G, Golden; LG, Light golden; DG, Dark golden; Y, Yellow; L, Lemon; W White.

calculated using the formula: % RSC = $((Ao_{Control} - Ai_{Sample}) / Ao_{Control}) \times 100\%$.

Determination of metal chelating activity

Fe^{+2} chelation of all extracts were analyzed by using a method as described by Dinis *et al*. (1994). 200 µl of extracts were added to a ferrous sulfate solution (2 mM, 500 µl). With the addition of ferrozine (0.25 mM; 500 µl), the reaction was started. After vigorously shaking it was kept at room temperature for 10 min. On the other hand, 200 µl of deionized water was used as a control to quantify the reaction mixture. Ferrozine reacted with divalent iron to form a stable magenta color complex which absorbs at 517 nm. The absorbance of the reaction mixture in the presence (A_i) and in the absence of extracts (A_o) was recorded. The absorbance of all extracts was also recorded and omitted from the Ai where needed. The percentage chelating activity of the extracts was calculated by formula as chelating rate (%) = $(A_o-A_i)/A_o \times 100$.

Antibacterial activity

Bacterial pathogens *viz*., *S. aureus, S. pyogene, S. epidermidis, P. aeruginosa, K. pneumonia*, *E. coli* and *S. marcescens* were isolated and identified in Biotechnology lab, The University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan (Awan *et al*., 2013). The antibacterial activity of all solvent extracts of *Z. officinale* and *A. sativum* were tested by agar disc diffusion method (Prescott *et al*.,1999). Similarly, the antibacterial activity of combined aqueous extracts of *Z. officinale* and *A. sativum* were also evaluated. The microorganisms were activated by inoculating a loop full of strain in 25 ml of nutrient broth medium and incubated at 37°C on a rotary shaker for 24 h. Next day, the old inoculated culture was mixed with freshly prepared nutrient agar medium when the temperature reached to 45°C and poured the sterilized plates. All plates were placed at room temperature in a laminar flow to solidify. The discs of 5 mm were prepared as follows: 200 µl of a particular extract was applied on disc and then allowed to dry for assay Presoaked discs were placed in Petri dishes at their labeled position. These prepared plates were left for incubation at 37°C for 48 h. Discs of diethyl ether, chloroform; ethanol and methanol were also used as a control. Before each experiment, spectrophotometry measured the optimal density (OD) of bacterial growth of 10^7 colony forming units (CFU)/ml at the wavelength of 600 nm (Seeley *et al*., 2001). The inhibitory effect was recorded by measuring the diameter

Table 2: Antibacterial activity of *A. sativum* and *Z. officinale* extracts against human bacterial pathogens

Extracts	*Klebsiella pneumoniae*	*Streptococcus pyogenes*	*Staphlococcus epidermidis*	*Staphlococcus aureus*	*Serratia marcesscens*	*Pseudomonas aeruginosa*	*Escherichia coli*
	Zone of inhibition of *A. sativum* {mm (Mean ±Standard deviation)}						
Aqueous (Aq)	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Diethyl Ether (Et)	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Chloroform (Ch)	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Ethanol (E)	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Methanol (M)	0±0	0±0	0±0	0±0	0±0	0±0	0±0
F.G.Et	11.33±1.15	31.00±1.00	16.68±7.68	31.33±1.58	4.00±1.00	3.67±0.58	5.00±1.00
F.G.Ch	16.00±1.73	31.67±2.08	12.00±2.00	23.67±3.21	10.00±2.00	2.67±1.58	4.67±1.15
F.G.E	2.00±1.00	31.67±1.58	16.33±1.58	31.67±2.88	11.67±1.58	3.67±0.58	2.00±1.00
F.G.M	1.33±0.58	35.00±1.00	31.33±1.15	40.00±1.00	21.67±2.88	3.00±1.00	1.33±0.58
F.G.Aq	12.00±1.53	7.67±2.51	31.00±1.00	33.00±2.64	36.67±2.88	8.00±1.00	11.00±1.00
N.D.G.Et	6.33±1.53	15.00±5.00	14.33±4.04	12.33±2.51	5.67±1.58	3.00±1.00	10.00±0.00
N.D.G.Ch	12.00±2.00	14.33±4.041	32.00±2.00	14.67±0.58	13.00±4.58	5.67±0.58	7.67±0.58
N.D.G.E	19.00±1.00	32.00±2.00	16.33±1.58	36.67±2.88	29.00±3.60	1.67±0.58	5.00±1.00
N.D.G.M	30.33±0.58	27.00±2.64	8.333±0.58	32.00±2.00	4.33±0.58	4.33±0.58	3.00±1.00
C.D.G.Et	8.33±0.578	22.67±2.57	0.00±0.00	1.33±0.58	4.33±1.58	1.33±0.58	9.67±1.58
C.D.G.Ch	6.00±1.00	8.00±2.64	6.00±1.00	4.00±1.00	10.33±2.08	3.00±0.00	12.33±0.58
C.D.G.E	11.67±1.58	15.67±2.08	2.00±1.00	1.00±0.00	14.00±1.00	8.00±1.00	3.00±1.00
C.D.G.M	8.33±0.58	34.67±0.58	4.33±0.58	0.00±0.00	3.00±1.00	4.33±0.58	2.00±1.00
	Zone of inhibition of *Z. officinale* {mm (Mean ±Standard deviation)}						
F.Z.Et	11.33±1.15	15.00±5.00	23.33±2.88	31.33±1.58	18.00±1.00	3.67±1.33	8.67±1.33
F.Z.Ch	17.00±1.00	36.33±2.08	30.67±0.58	31.67±1.58	6.00±2.64	7.00±1.00	11.00±1.00
F.Z.E	4.67±0.58	7.67±2.57	4.33±0.58	2.00±1.00	15.00±1.00	11.33±1.33	4.00±1.00
F.Z.M	10.33±0.58	26.33±1.58	1.33±0.58	2.67±1.58	25.00±5.00	10.00±1.00	2.00±1.00
F.Z.Aq	8.33±0.58	11.67±2.88	6.67±0.58	21.67±2.08	32.00±2.00	5.33±1.33	12.33±0.33
N.D.Z.Et	8.00±1.00	22.00±2.00	4.00±1.00	38.33±1.58	9.67±2.08	3.33±0.33	14.00±1.00
N.D.Z.Ch	13.33±2.08	8.33±2.88	8.00±1.00	32.33±2.08	6.33±1.58	2.33±0.33	10.33±0.33
N.D.Z.E	10.33±0.58	13.33±2.88	0.00±0.00	35.67±2.08	3.00±1.00	2.00±1.00	3.00±1.00
N.D.Z.M	17.33±2.57	14.00±1.00	2.00±1.00	27.67±2.51	2.00±1.00	5.00±1.00	13.67±10.33
C.D.Z.Et	2.67±0.58	32.00±2.00	11.00±3.60	23.33±2.88	5.67±1.58	2.00±1.00	1.33±0.33
C.D.Z.Ch	3.67±0.58	30.00±1.00	18.00±2.00	21.67±1.58	6.67±1.58	2.33±1.33	2.00±1.00
C.D.Z.E	11.67±2.08	9.00±1.00	5.00±1.00	20.67±1.58	4.33±1.58	5.00±1.00	1.33±0.33
C.D.Z.M	7.00±1.00	7.00±1.00	8.00±1.00	30.33±1.58	9.67±1.58	3.67±1.33	2.00±1.00

The results of sensitivity tests were expressed as (0) for no sensitivity, (below 12 mm) for low sensitivity, (12 to 29 mm) for moderate sensitivity and (30 to 45 mm) for high sensitivity.

of the zone of inhibition after 24-48 h in millimeter (mm). The results of sensitivity tests were expressed as (0) for no sensitivity, (below 12 mm) for low sensitivity, (12 to 29 mm) for moderate sensitivity and (30 to 45 mm) for high sensitivity. The Sensitivity of standard antibiotics against test microbial strains was also assessed through agar disc diffusion method (Natta *et al*., 2008).

Phytochemical screening

Qualitative screening of plants extracts

The powdered extracts of *A. sativum* and *Z. officinale* were evaluated for qualitative determination of major phytoconstituents i.e. cardic glycosides, flavonoids, alkaloids, tannins, saponins and steroids. The phytochemical tests were performed according to describe methods (Awan *et al*., 2013; Sofowora *et al*., 1993; Iyengar, 1985)

Total phenolic content

Phenolic contents (mg/100 ml of extracts) were determined with slight modifications, using the Folin-Ciocalteu reagent method described by Zhou and Yu, (2006). The reaction mixture was made of *A. sativum* and *Z. officinale* extracts (100 µl), Folin-Ciocalteu reagent (100 µl), and 20% sodium carbonate (3 ml). The reaction mixture was incubated at room temperature for 1h and the absorbance of the deep blue complex was measured at 765 nm. Gallic acid was used as a standard with varied concentration from 200 ppm to 1000 ppm. The total phenolic contents were expressed as mg gallic acid equivalents per gram extract weight (mg/100 gm).

Total flavonoid content

Estimation of total flavonoid contents of extracts was quantified by the method illustrated by (Zou *et al*., 2004). The reaction mixture cocktail containing extracts (500µl), distilled water (2 ml), and 5% $NaNO_2$ (0.15 ml) was prepared and incubated at room temperature for 6 min. After incubation, 10% $AlCl_3$ (0.15 ml) solution was added, kept further for 6 min at room temperature, followed by the addition of 2 ml of 4% NaOH solution. After the addition of water to sample to make the final

Table 3: Comparative analysis of antibacterial activities of commercial and natural dried *Z. officinale* and *A. sativum*

Natural *A. sativum* and *Z. officinale*		Commercial *A. sativum* and *Z. officinale*		Pathogens
Zone of inhibition in mm (Mean ±Standard deviation)				
GZN	30.67±0.58	GZC	10.00±1.00	*Klebsiella pneumoniae*
GZN	32.00±2.00	GZC	2.00±1.00	*Streptococcus pyogenes*
GZN	4.33±0.58	GZC	4.33±0.58	*Staphlococcus epidermidis*
GZN	37.00±1.73	GZC	16.00±1.00	*Staphlococcus aureus*
GZN	31.67±2.08	GZC	20.00±1.00	*Serratia marcesscens*
GZN	33.67±1.53	GZC	13.00±1.00	*Pseudomonas aeruginosa*
GZN	16.00±1.00	GZC	5.67±0.58	*Escherichia coli*

The results of sensitivity tests were expressed as (below 12 mm) for low sensitivity, (12 to 29 mm) for moderate sensitivity and (30 to 45 mm) for high sensitivity.

Table 4: Phytochemical analysis of *A. sativum* and *Z. officinale.*

Chemical compounds	Observations	*A. sativum*			*Z. officinale*		
		F.G	N.D.G	C.D.G	F.Z	N.D.Z	C.D.Z
Cardic glycoside	Brownish ring	+	+	+	+	+	-
Flavonoids	Yellow	+	+	-	+	+	+
Tannins	Brownish green	+	+	+	+	+	+
Saponins	Forth formation	+	+	+	+	+	-
Alkaloids	Brown precipitate	+	+	-	+	+	+
Steroids	Green	+	+	+	+	+	+
Phenols	Gray color	+	+	+	+	+	+

(+ Presence, - not detected)

Table 5: Quantitative phytochemical analysis of *A. sativum* and *Z. officinale.*

Sample	Phenols (mg of GAE/g)	Flavonoids (mg of RUE/g)	Tannins (mg of TAE/g)
A. sativum			
F.G	17.40 ±1.2	3.39 ±1.2	2.55 ±0.3
N.D.G	10.05 ±0.5	3.05 ±0.1	2.00 ±0.1
C.D.G	12.52 ±0.3	3.01±0.9	2.11±0.9
Z. officinale			
F.Z	12.13 ±2.25	6.66 ±0.8	4.51 ±1.2
N.D.Z	9.39 ±0.81	5.59 ±0.6	3.10.00 ±0.7
C.D.Z	10.01 ±0.92	6.61±0.7	2.99±0.9

GAE (milligrams of Gallic Acid equivalent); RUE (milligrams of Rutin equivalent); TAE (milligrams of Tanic Acid equivalent)

volume to 10 ml, the mixture was mixed immediately and kept for 15 min. The absorbance of the reaction mixture was measured at 510 nm. Rutin was used as a standard for the calibration curve. Total flavonoid contents of extracts and fractions were expressed as mg rutin equivalents (RE) per gram of sample (mg/g).

Total tannin estimation

Estimation of tannins is based on the measurement of blue color produced by the reduction of phosphotungstomolybdic acid by tannin-like compounds in alkaline solution (Ranganna, 1986). The extract (200 µl) was mixed with 5.0 ml of Folin- Denis reagent (FD) and Na_2Co_3 solution, mixed well and after 30 min of incubation at the room, temperature absorbance was recorded at 760 nm using a spectrophotometer. Total tannin contents were as expressed as mg tannic acid equivalent /100 g of sample.

Chemical screening through thin layer chromatography

The indication of major phytochemicals was further evaluated by TLC using precoated Silica gel 60F264 plates (Wagner and Bladt, 2004). In order to get better resolution of components, different screening systems were used. The developed plates were observed using various reagents (anisaldehyde/ H_2SO_4) and UV light (254-336 nm). R_f value of each spot was calculated as R_f = distance traveled by the solute/ distance traveled by the solvent.

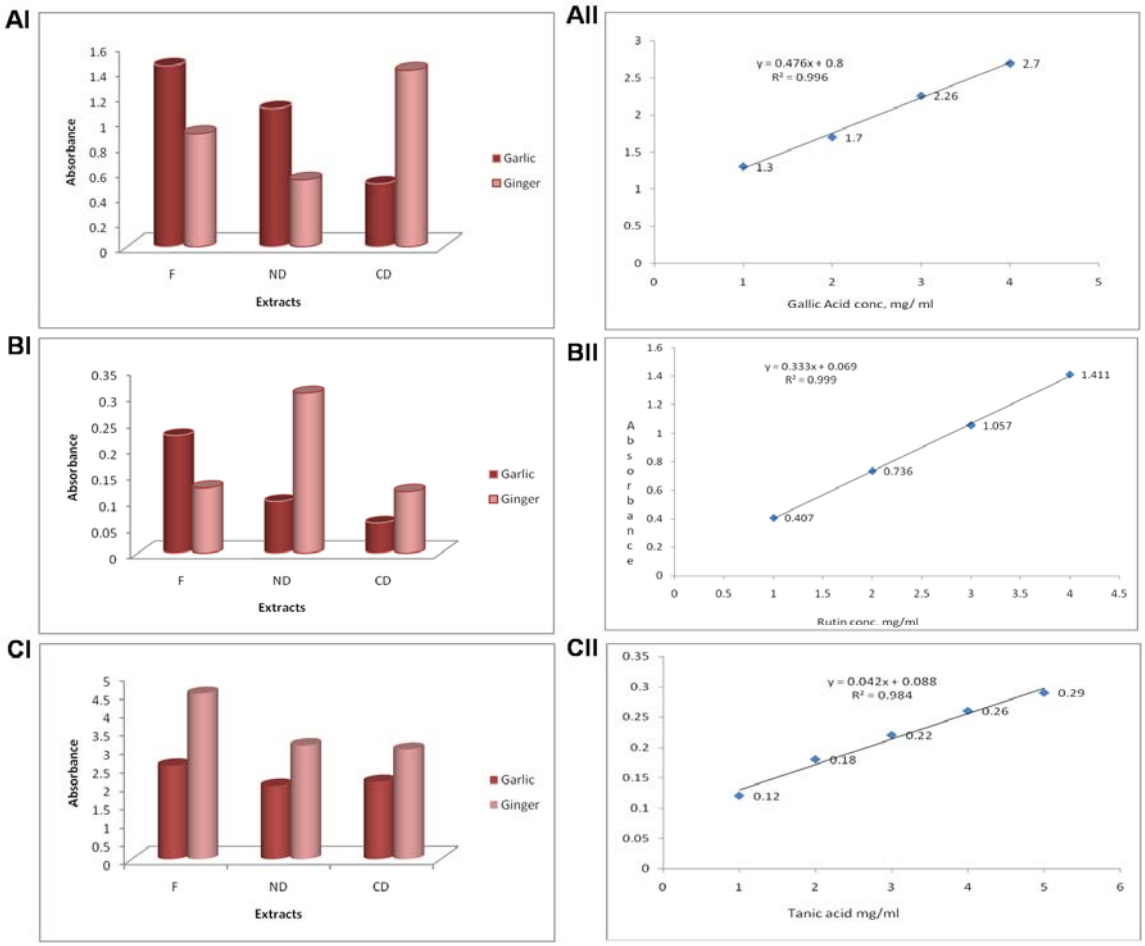

Fig. 2: Quantitative analysis of total phenolics, flavonoids and tannins in both *A. sativum* and *Z. officinale*. (A.I) Gallic acid equivalent phenolic content in mg/100 ml of *A. sativum*, and *Z. officinale* extracts absorbance recorded at 765 nm. (A. II) Calibration Curve using gallic acid as a standard. (B. I) Rutin equivalent flavonoids content in mg/100 ml of *A. sativum* and *Z. officinale* extracts absorbance recorded at 510 nm. (B. II) Calibration Curve using rutin as a standard. (C. I) Tanic acid equivalent tanins content in mg/100 ml of *A. sativum,* and *Z. officinale* extracts absorbance recorded at 510 nm. (C. II) Calibration Curve using tanic acid as a standard. F, fresh; ND, naturally dried; CD, commercially dried.

Biological screening through direct bioautography

To measure the direct bioautography, agar overlay technique was used with slight modifications as demonstrated by Slusarenko *et al.* (1998). The multi-drug resistant strain of *S. aureus* was taken. 10 ml of plant extract was used to spot on the silica gel plates. For separation chloroform and methanol 1:1 was used as a solvent system. The developed chromatogram was placed in sterilized Petri plates. The fresh overnight culture of *S. aureus* was mixed with freshly prepared nutrient agar and then poured over the chromatogram as a thin layer. The plates were left at room temperature for 5 min and then incubated overnight at 37°C. The zone of growth inhibition (GI) was recorded around the active chromatogram spot.

FTIR spectroscopy analysis

FTIR spectra were collected using a Perkin Elmer FTIR spectrometer (100 series). The aluminum oxide membrane filter, coated with a uniform thin layer of powder extract, was placed in direct contact with the diamond crystal cell (30,000 to 200 cm^{-1}) of attenuated total reflectance (ATR). Infrared spectra were recorded from 4,000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. Four spectra were acquired at room temperature for each sample. Triplicate experiments were conducted.

STATISTICAL ANALYSIS

Each experiment was repeated in triplicates and Mean ± Standard Deviation from absolute data was calculated. The comparison of the antibacterial activity of *A. sativum* and *Z. officinale* extracts with that of standard antibiotics was determined by activity index (AI) (Shekhawat and

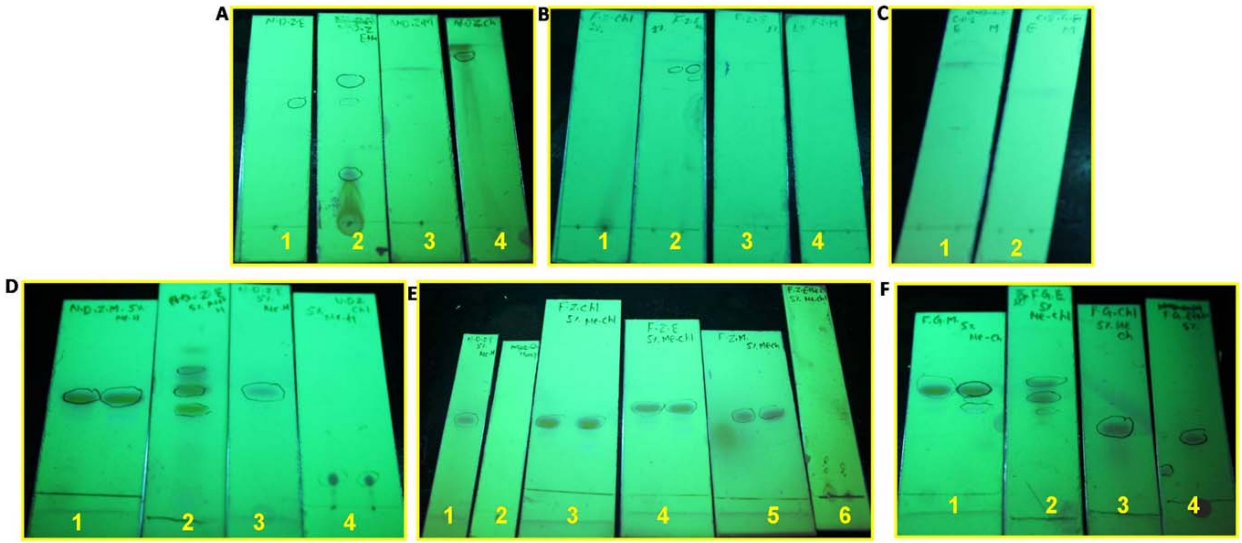

Fig. 3: Thin layer chromatography of *A. sativum* and *Z. officinale* Extracts. A: solvent system $CHCl_3$: MeOH (9.9: 0.1), 1= Naturally dried *Z. officinale* in ethanol, 2= Naturally dried *Z. officinale* in ether, 3= Naturally dried *Z. officinale* in methanol, 4= Naturally dried *Z. officinale* in chloroform; B: solvent system $CHCl_3$: MeOH (9.9: 0.1), 1= fresh *Z. officinale* in chloroform, 2= fresh *Z. officinale* in ether, 3= fresh *Z. officinale* in ethanol, 4= fresh *Z. officinale* in methanol); C: solvent system $CHCl_3$: MeOH (9.9: 0.1), 1= commercially dried *Z. officinale* in ether and commercially dried *Z. officinale* in chloroform, 2= commercially dried *Z. officinale* in ethanol and commercially dried *Z. officinale* in methanol; D: solvent system $CHCl_3$: MeOH (9.5: 0.5), 1= Naturally dried *Z. officinale* in methanol, 2= Naturally dried *Z. officinale* in ethanol, 3= Naturally dried *Z. officinale* in ether, 4= Naturally dried *Z. officinale* in chloroform; E: solvent system $CHCl_3$: MeOH (9.5: 0.5), 1= commercially dried *Z. officinale* in ethanol, 2= commercially dried *Z. officinale* in methanol, 3= fresh *Z. officinale* in chloroform, 4= fresh *Z. officinale* in ethanol, 5= resh *Z. officinale* in methanol), 6= fresh *Z. officinale* in ether; F: solvent system $CHCl_3$: MeOH (9.5: 0.5), 1= fresh *A. sativum* in methanol, 2= fresh *A. sativum* in ethanol, 3= fresh *A. sativum* in chloroform, 4= fresh *A. sativum* in ether.

Vijayvergia, 2010). For measuring activity index, following formula was used {Activity index= zone of inhibition of extract / zone of inhibition of antibiotic}. Data was further treated by analysis of variance (ANOVA) through http://www.danielsoper.com/statcalc3/calc.aspx?id=43. Differences among means were determined by the least significant difference (LSD) test with significance defined at $P < 0.05$ using SPSS version 13.0 software. Calibration curve and regression coefficient lines were drawn in Microsoft Excel. Spectra of FTIR were mapped using origin version 6.1 software.

RESULTS

Physical properties of A. officinale and A. sativum in various solvents

Most of the extracts of *A. sativum* and *Z. officinale* were pasty in nature with a characteristic smell and having different colors such as golden, light and dark golden, lemon, light and dark lemon, yellow, pale yellow and light yellow, transparent and colorless (table 1). Mostly naturally dried *A. sativum* (NDG) in solvent extracts were colorless whereas naturally dried *Z. officinale* NDZ showed golden color. *Z. officinale* extracts have more pungent odor than *A. sativum*. Fresh extracts were pasty in nature.

Antioxidant activity of extracts of A. sativum and Z. officinale

Antioxidant potential of extracts was measured by $ABTS^+$ decolorization assay. Naturally and commercially dried *A. sativum* extracts (N.D.G and C.D.G) of chloroform, ethanol, methanol and fresh aqueous (F.G) possessed stronger $ABTS^+$ scavenging potential, with potential values from 89% to 96%. Similarly, methanol and aqueous extracts of fresh *A. sativum* (F.G) showed 95 %and 90% scavenging activity. However, the scavenging activity of a fresh extract of *Z. officinale* (F.Z) was too low except aqueous extract. Commercially dried extracts (C.D.Z) have stronger activity whereas naturally dried extracts (N.D.Z) also showed reasonable activity. The free radical scavenging potential values of *Z. officinale* fresh extracts was 8, 15, 23, 55 and 88%, naturally dried *Z. officinale* was 94, 40, 43, 46 and 55% and commercially dried extracts of *Z. officinale* was 94, 91, 68, 89 and 47% respectively. Combined effect of *A. sativum* and *Z. officinale* showed considerable results with values of 58, 81, and 94% of natural extracts and 51, 59, and 90% of commercial extracts. On the other hand, the combined effect of natural *A. sativum* and *Z. officinale* (G+Z) was greater 92% than commercial *A. sativum* and *Z. officinale* 75%. It was found that *Allium sativum* and *Z. officinale* extracts are excellent sources of antioxidant.

Metal chelating of Z. officinale and A. sativum

All extracts of *A. sativum* and *Z. officinale* showed stronger metal chelation potential. Comparison of the effect of incubation time of metal ion chelating potential of the extracts revealed that after incubation metal chelating potential of all *A. sativum* extracts was considerably increased. On the other hand, the metal chelating potential of all *Z. officinale* extracts was considerably decreased after 24 min of incubation. While the extract of C.D.Z showed same behavior after both 15 and 24 min of incubation.

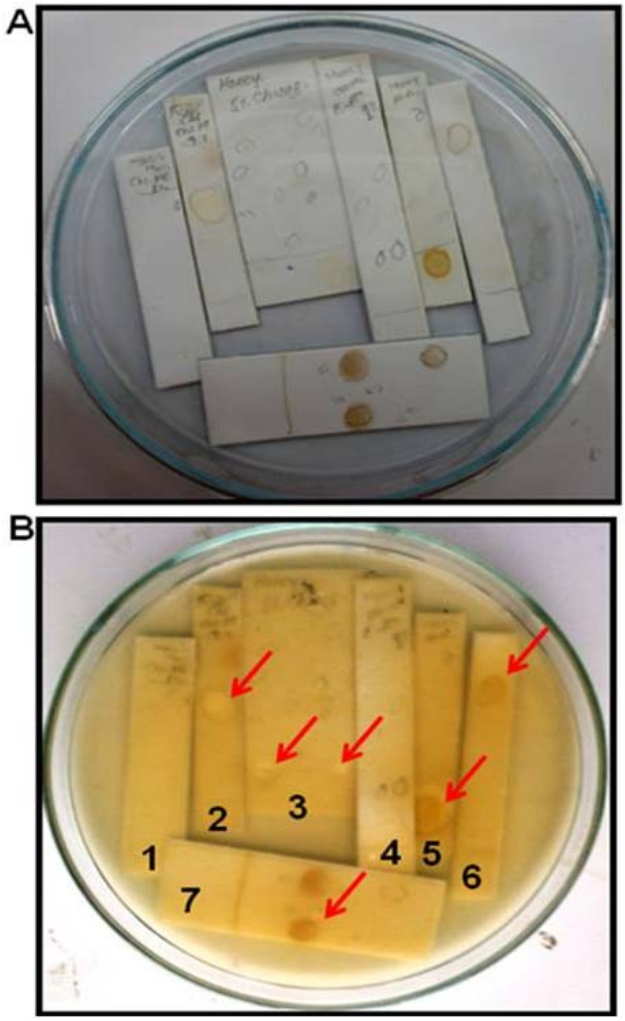

Fig. 4: Bioautography of *A. sativum* and *Z. officinale* extracts against *S. aureus*. (1= naturally dried *A. sativum* extract in methanol; 2= naturally *A. sativum* extract in chloroform; 3= fresh *A. sativum* extract in methanol; 4= naturally *Z. officinale* extract in ethanol; 5= naturally dried *Z. officinale* extract in ethanol; 6= naturally dried *Z. officinale* extract in methanol; 7= fresh *Z. officinale* extract in methanol).

Antibacterial activity of A. sativum

Antibacterial activity of *A. sativum* was observed against human-associated bacterial pathogens through agar disc diffusion methods. It was observed that methanolic extract of naturally dried *A. sativum* (N.D.G.M) indicated highly significant activity (30.33 mm) against *K. pneumoniae*, whereas chloroform and ethanolic extracts of both fresh and naturally dried *A. sativum* (F.G.Ch and N.D.G.E) showed moderate inhibitory results (16 and 19 mm; table 2). All fresh extracts of *A. sativum* such as F.G.Et, F.G.Ch, F.G.E, F.G.M except aqueous (F.G.Aq), N.D.G.M, N.D.G.E and C.D.G.M indicated highly significant zone of inhibition against *S. pyogenes* (31, 31, 31, 35, 27, 32 and 34 mm), whereas all other extracts of *A. sativum* showed low sensitivity against *S. pyogenes*. Similarly, F.G.M, F.G.Aq and N.D.G.Ch indicated significant inhibition of 31, 31 and 32 mm against *S. epidermidis* whereas F.G.Et, F.G.E and N.D.G.E indicated moderate inhibition of 16.6, 16.3, and 16.3mm (table 2). The extracts of commercially dried *A. sativum* did not show inhibition of *S. epidermidis.* On the other hand, all fresh extracts of *A. sativum* except chloroform (F.G.Et, F.G.E, F.G.M, F.G.Aq), N.D.G.E and N.D.G.M showed strong zone of inhibition against *S. aureus* (31.3, 31.6, 40, 33, 36 and 32 mm) as compared to other extracts while F.G.Ch showed moderate inhibition (23.66mm). In a case of *S. marscesnes* N.D.G.E and F.G.Aq indicated strong inhibition of 36.66 and 29mm whereas F.M showed a moderate zone of inhibition (21.66mm). Interestingly all solvent extracts of *A. sativum* showed low sensitivity against *P. aeruginosa*. Similar results were also observed in the case of *E. coli* (table 2). This indicated a broad spectrum of activity of naturally dried and fresh *A. sativum* extracts against both Gram-positive and Gram-negative bacteria (fig. 1).

Antibacterial activity of Z. officinale

Antibacterial activity of *Z. officinale* was analyzed against human associated bacterial pathogens through agar disc diffusion methods. All extracts of *Z. officinale* showed low sensitivity against *K. pneumoniae* except F.Z.Ch and N.D.Z.M (17 and 17.33mm) showed moderate inhibition. On the other hand, chloroform, methanolic and diethyl ether extracts of fresh and commercially dried *Z. officinale* (F.Z.Ch, F.Z.M, C.D.Z.Et and C.D.Z.Ch) indicated highest zone of inhibition against *S. pyogenes* (36.3, 26.33, 32 and 30 mm) as compared to other extracts while diethyl ether extract of naturally dried *Z. officinale* (N.D.Z.Et) showed moderate inhibition (22 mm) of *S. pyogenes* (table 2). Similarly, fresh extracts of *Z. officinale* in chloroform solvent indicated stronger inhibition of *S. epidermidis* (30.66 mm) and F.Z.Et showed moderate activity (23.33 mm) whereas all other extracts showed low sensitivity against *S. epidermidis* range between 1-15 mm (table 2). Interestingly it was observed that all extracts of naturally dried *Z. officinale* showed a stronger inhibitory effect against *S. aureus* i.e. 30, 32.33, 35.66 and 27.6 mm whereas commercial extracts of *Z. officinale* showed moderate results i.e 23.33, 21.6 and 20 mm. In the same way, F.Z.Et, F.Z.Ch, and C.D.Z.M showed also a strong zone of inhibition against *S. aureus* (31.3, 31.6, 31.3 mm). All extracts of fresh and

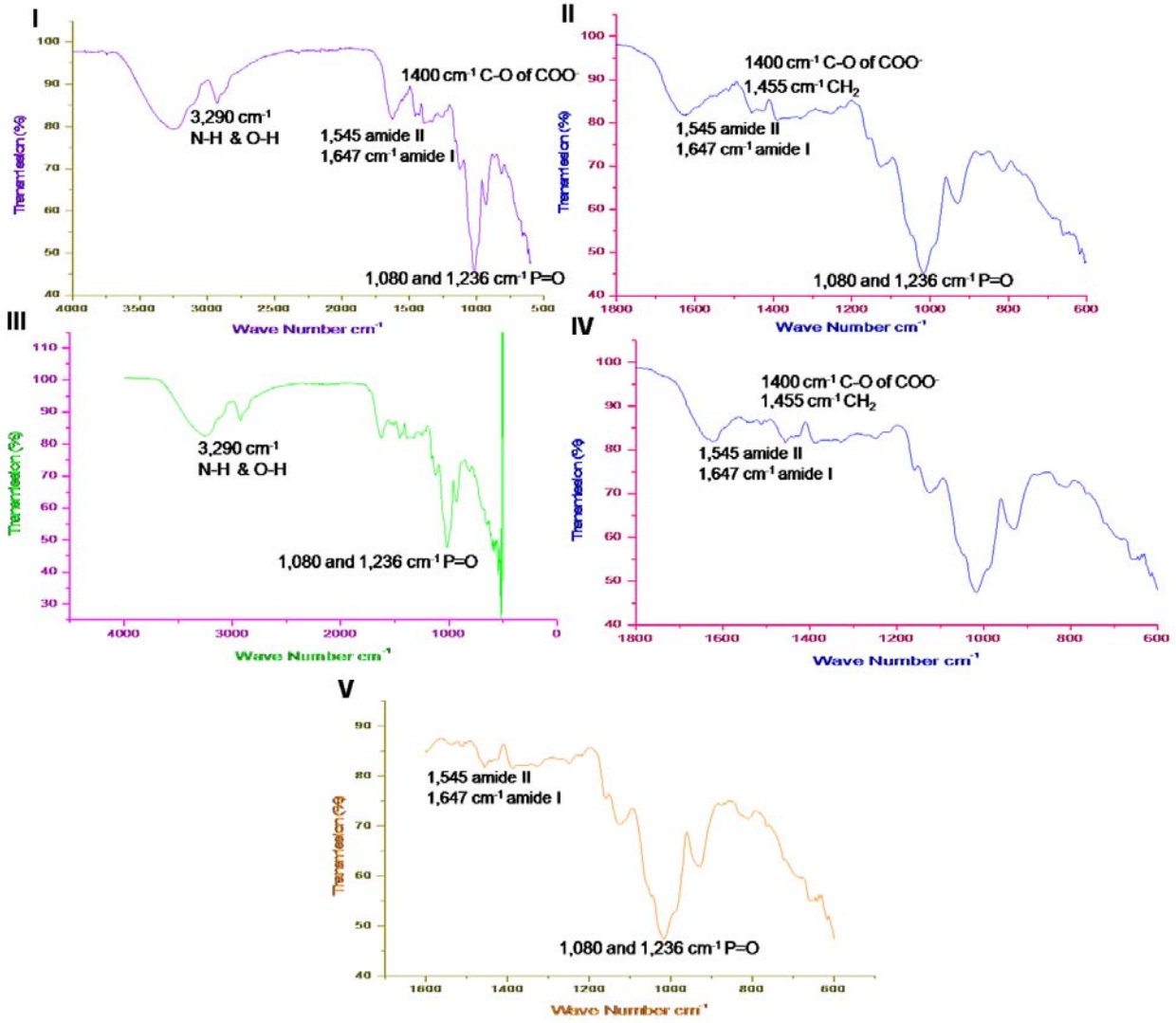

Fig. 5: FTIR spectrometry of natural and commercial *A. sativum*. I-III indicate the spectrum of natural *A. sativum*; IV and V- indicates the spectrum of commercial *A. sativum.*

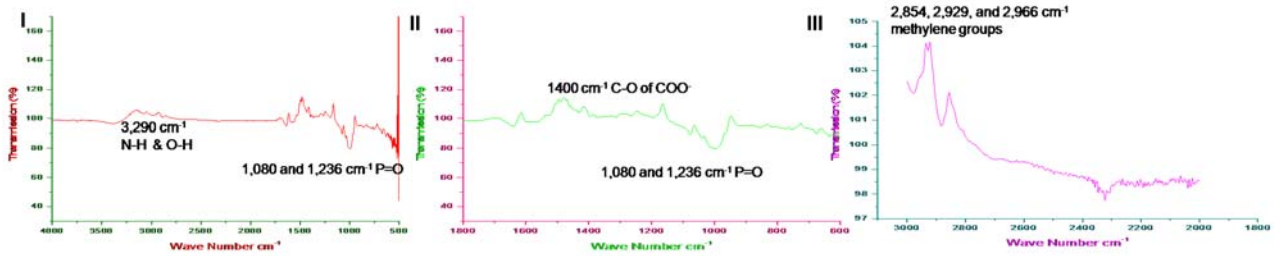

Fig. 6: FTIR spectrometry of natural and commercial *Z. officinale*. Both natural and commercial *Z. officinale* produced

natural and commercial dried *Z. officinale* showed low inhibition against both *P. aeruginosa* and *E. coli* (table 2). In the case of antibacterial activity of *Z. officinale* it has been concluded that diethyl ether and chloroform extracts showed a greater zone of inhibition against tested pathogens except for *P. aeruginosa* and *E. coli* (fig. 1). Maximum zone of inhibition was recorded against *K. pneumoniae, S. pyogenes, S. marcescens, P. aeruginosa,* and *S. aureus* (30.66, 32, 37, 31.66, and 33.66 mm) when the combination of both aqueous extracts of natural *A. sativum* and *Z. officinale* (G.Z.N) was applied in 1:1 ratio whereas moderate inhibition was shown by *E. coli* (16 mm). On the other hand, combined extracts had no effect on *S. epidermidis* (table 3). In the same way, moderate effect was recorded against *S. aureus* and *S. marcescens* (16 and 20 mm) when combination of aqueous extracts of

both commercial *Z. officinale* and *A. sativum* (G.Z.C) was applied in ratio 1:1 whereas no antibacterial activity was observed against *K. pneumoniae, S. pyogenes, S. epidermidis, P. aeruginosa,* and *E. coli* (table 3). Interestingly it was found that combined extract of both natural and commercial *Z. officinale* and *A. sativum* had low effect on *S. epidermidis* (table 3).

Activity index (AI) analysis against pathogens

Activity index of both *A. sativum* and *Z. officinale* extracts with various antibiotics were determined against all tested pathogens. In the case of *K. pneumoniae*, the results demonstrated that all extracts showed strong activity than all tested antibiotics except penicillin G. against *S. pyogenes*, all extracts showed very high activity than oxytetracycline, sulfamethoxazole, tetracycline, penicillin G, trimethoprim, ampicillin, and amoxicillin. We found that these extracts had a stronger antibacterial effect compared to recommended antibiotics. The most significant activity of all extracts was observed against *S. epidermidis* except C.D.G.Et of *A. sativum* and N.D.Z.E extract of *Z. officinale*. All extracts of *A. sativum* and *Z. officinale* showed the strongest activity than neomycin, penicillin G, amoxicillin. In the case of *S. aureus* all extracts showed the highest activity than oxytetracycline, sulfamethoxazole, tetracycline, penicillin G, ampicillin, and amoxicillin. All fresh, as well as ethanolic and methanolic extracts of N.D.G showed higher activity vancomycin, chloramphenicol, streptomycin, tobramycin, neomycin, trimethoprim, and kanamycin. Whereas C.D.M extract of *A. sativum* had no effect against *S. aureus*. All extracts of *Z. officinale* and *A. sativum* showed the the highest activity against *S. marscences* than tetracycline.

All extracts of *A. sativum* showed less activity against *P. aeruginosa* than all tested antibiotics except ampicillin and vancomycin. Chloroform extract of N.D.G and ethanolic extract of C.D.G showed good activity than oxytetracycline, sulfamethoxazole, tetracycline, penicillin G and amoxicillin. In the case of *E. coli,* all tested extracts of *Z. officinale* showed stronger activity than vancomycin, gentamicin, oxytetracycline, sulfamethoxazole, penicillin G, trimethoprim, amoxicillin, and ampicillin. Considerably stronger activity was reported than tobramycin and tetracycline. F.Z.E, N.D.Z.Ch and N.D.Z.M showed higher activity than ciprofloxacin, nalidixic acid, neomycin, and kanamycin. From activity index analysis, we found that this *A. sativum* and *Z. officinale* extracts had potential compounds that showed strong inhibition of tested pathogens.

Phytochemical analysis of A. sativum and Z. officinale

The data of qualitative determination of secondary metabolites of *A. sativum* was tabulated in table 4. Among the seven groups of phytochemicals determined from the fresh, naturally dried and commercially available *A. sativum* glycosides, tannins and alkaloids were found to be the most abundant in fresh and N.D.G one followed by saponins, and flavonoids. While steroids were low in concentration and anthraquinone were absent. In the case of C.D.G except for flavonoids, which were absent, all other tested phytochemicals were present in low concentration. In the case of *Z. officinale,* flavonoids and saponins were present in high concentration in F.Z and N.D.Z. followed by tannins, steroids, and carbohydrates which were moderately present, glycosides and alkaloids were present in low concentration. In the case of C.D.Z., flavonoids and steroids were reasonably present, tannins, alkaloids, and carbohydrates were present in low concentration while, glycosides, saponins, and anthraquinone were absent (table 4).

When *A. sativum* and *Z. officinale* were quantitative analyzed we found that the total phenolic, flavonoid and tannin contents in the fresh extracts were more than in the naturally dried, followed by contents in the commercially available powders (table 5). Comparing the extracts of tested plants, it was found that fresh *A. sativum* had higher contents of total phenols (17.4) than fresh *Z. officinale* (12.1) mg gallic acid equivalent /100g of the sample with reference to a standard curve (Y=0.476x+0.8, r2=0.996) (fig. 2A). While fresh *Z. officinale* had higher total flavonoids (6.66) than fresh *A. sativum* (3.39) mg rutin equivalent /100 g of sample with reference to a standard curve (Y=0.333x+0.069, r^2=0.999) (fig. 2.B). The amount of total tannins in *Z. officinale* extracts was higher (4.51) than *A. sativum* extracts (table 5) mg tannic acid equivalent /100g of the sample with reference to a standard curve (Y=0.0042x+0.088,r2=0.984) (fig. 2C). Differences between two plants and also the three different types of extracts of plants (fresh, naturally dried and commercially dried) were highly significant ($p<0.002$).

Thin layer chromatography of A. sativum and Z. officinale extracts

In chemical screening performed by TLC on the crude extracts of *A. sativum* and *Z. officinale* an impressive diversity of the chemical constituents can be seen (fig. 3). In TLC analysis, different absorbing bands were observed under short and long wavelength of UV i.e., 254 nm and 336 nm, respectively. Prominent colored bands of different crude extracts were observed by staining with anisaldehyde/ H_2SO_4 and also observed under UV. The colored bands like red, orange, brownish to yellow, red to brown were indicating the presence of different types of amines etc. Similarly, yellow, purple, blue and brown colored bands were indicating the presence of the functional groups like phenols, steroids, terpenes, flavonoids in the crude extracts. R_f value of each spot was calculated, where R_f = Distance of the spot from start point/ Distance of solvent front from start point. In the case of *A. sativum*, the most promising diversity of the colored bands was seen in the crude ethanolic and methanolic extracts of the F.G and N.D.G in the chloroform: methanol solvent system, while C.D.G extracts showed no result on TLC plates. Similarly in

ethyl acetate: Acetic acid system, the crude ethanolic and methanolic extracts of F.G and N.D.G showed prominent colored bands. In the case of *Z. officinale* extracts, the most significant diversity of the colored bands was observed in all solvent extracts of F.Z and N.D.Z in both chloroform: methanol and ethyl acetate: acetic acid systems, whereas C.D.Z showed no separation. In *n*-hexane: methanol solvent system significant colored bands were seen except in C.D.Z. On the other hand, in *n*-hexane: ethyl acetate and *n*-hexane: Acetone systems both ethanolic and methanolic extracts showed significant colored bands, while other extracts remained inactive

Bioautography of A. sativum and Z. officinale

The results of bioautography of *A. sativum* and *Z. officinale* extracts against the multi-drug resistant strain of S. *aureus* revealed significant activity (fig. 4). Fresh extracts of both *A. sativum* and *Z. officinale* showed stronger activity on chromatogram as compared to dried extracts. Maximum zone of inhibition was recorded by ethanolic and methanolic extracts of fresh *A. sativum* and *Z. officinale*. In fig. 4 arrows represent the appearance of visible zones of inhibition exerted by extracts of *A. sativum* and Z. *officinale*.

FTIR spectrometry

The complementary (IR) spectral features provided more useful information about the biochemical components of the *A. sativum* and *Z. officinale* extracts (fig. 5 and 6). FT-IR spectroscopy provided a "fingerprint" region below the wave number of 1800cm^{-1}, which reflects detailed information about the composition of extracts. For FT-IR spectra, the distinctive band at 3290, 2854, 2929, 2966, 1545 and 1647, 1455, 1400, 1080 and 1236 cm^{-1} is assigned to the N-H stretch of proteins, O-H stretch of polysaccharides and water, methylene groups from lipids, amide II and amide I, the secondary structure of protein, CH_2 bending of lipids, symmetric stretch of C-O of COO^- groups, symmetric and antisymmetric stretch of P=O of nucleic acids, respectively.

DISCUSSION

Pharmacological analysis of A. sativum

A. sativum has a significant effect on food-borne pathogenic bacteria *viz*., *Shigella, Salmonella*, and *Staphylococcus* (Teferi and Hahn, 2002). *A. sativum* extracts can also prevent the formation of *Staphylococcus* enterotoxins A, B, and C1 (Gonzalez-Fandos *et al*., 1994). Our results are consistent with the interpretation of previous literature (Shokradeh and Ebadi, 2006) and it has been proved that *A. sativum* may be used successfully for treating *S. aureus* and other infectious pathogens (Daka, 2011). This could be due to the presence of cardiac glycosides as revealed by this study as confirmed by TLC. *A. sativum* have high ranging from antibacterial (Akiyama *et al*., 2001) to antiparasitic activities (Herbert and Albrecht, 2005). Allicin (Friedman *et al*., 2002), diallyl sulfides (O'Gara *et al*., 2000), and ajoene (Naganawa *et al*., 1996) are principally responsible for the bioactive properties. Organosulfur compounds and polyphenols have both antimicrobial and antioxidant activity and these two properties are related to each other. Most of these antimicrobial agents present in *A. sativum* alter microbial cell membranes causing leakage or autolysis consequently inhibiting growth or causing cell death (Yin and Cheng, 2003).

In vitro antimicrobial activity of *A. sativum* was also observed against *E. coli, B. subtilis*, and *S. cerevisiae* (De *et al*., 1999). Similarly, the significant bactericidal effect of extracts of *A. sativum* was observed against *S. typhimurium, S. epidermidis*, and various yeasts (Arora and Kaur, 1999). Antibiotic-resistant bacteria were also sensitive to *A. sativum* extracts (Indu *et al*., 2006). *A. sativum* and *Z. officinale* did not exhibit *in vitro* inhibition on test organisms including *Staphylococcus* spp. when applied singly or in combination (Slusarenko *et al*., 1996). In contrast, our study has clearly shown that *S. aureus* was inhibited by these medicinal plants. Similarly, Vuddhakul *et al*. (2007) also clearly indicated the inhibition of *S. aureus, E. coli* and *V. parahaemolyticus* by *A. Sativum* extracts. The ethanolic extracts of *A. sativum* did not have an effect on *E. coli* and *Shigella* consistent with Ahmad and Aqil, (2007). Earlier studies had reported that 3%, 5%, and 10% fresh *A. sativum* extracts were not inhibited *B. subtilis* and *P. aeruginosa* (Saleem and Al-Delaimy, 1982). It was indicated that the crushed *A. sativum* contains a class of volatile sulfur compounds called thiosulfonates, of which allicin is the most frequent and responsible for its various activities like antibacterial (Deshpande *et al*., 1993). Similarly, flavonoids and phenolic molecules have been demonstrated to have direct antimicrobial activity against important clinical isolates including *P. aeruginosa, Salmonella spp., E. coli*, *Enterobacter spp., Klebsiella spp., Helicobacter pylori*, methicillin resistant *S. aureus* (MRSA) and pathogenic fungi by inhibiting nucleic acid 138 synthesis, energy metabolism or by disrupting cell membrane function (El-Gendy *et al*., 2008). Our results also showed similar results as obtained from an ethanolic extract of *Z. officinale* (Ibrahim, 2010). It has been concluded that mostly ethanol and methanol extracts of *A. sativum* showed greater and significant inhibition of all tested pathogens except *P. aeruginosa* and *E. coli*. The reason behind is that ethanol and methanol are polar solvents they can dissolve all polar compounds present in extracts, so researchers say that there might be some polar compounds in *A. sativum* having reasonable antibacterial potency. This may be due to the lipid content of membranes of different groups of microorganisms and permeability of allicin and other *A. sativum* constituents.

Pharmacological analysis of Z. officinale

Z. officinale belongs to family *Zingiberaceae* is widely used as spices and food preservation. Yu *et al*. (2007) demonstrated that *Z. officinale* are added to food products as essential oils and in the form of various extracts. It has been working as a folk medicine to against several diseases, and illnesses such as dyspepsia, blood circulation disturbance, gastritis, and inflammatory diseases (Geiger, 2005). The probable antiallergenic, analgesic, antipyretic and chemopreventive activities of *Z. officinale* were also studied (Gurdip *et al*., 2008). Fresh *Z. officinale* contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fiber and 12.3% carbohydrates. The minerals present in *Z. officinale* are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C. The composition varies with the type, variety, agronomic conditions, curing methods, drying and storage conditions (Govindarajan, 1982).

The inhibition activity of *Z. officinale* extracts could be attributed to the chemical properties of *Z. officinale*. The main constituents of *Z. officinale* are sesquiterpenoids with zingiberene as the main component. Other components include β-sesquiphellandrene, bisabolene, and farnesene, which are sesquiterpenoids, and trace monoterpenoid fraction, (β-sesquiphellandrene, cineol and citral) pungent substances namely gingerol, shogaol, zingerone and paradol (Wang *et al*., 2009).

Our results are in superior consistency with the conclusions of various researchers and also compared with several other investigations (Ali *et al*., 2005). In the current research, it had found that *Z. officinale* extracts exhibited an inhibitory effect against pathogenic bacteria and their effect was possibly due to the presence of their main components. These components were identified with the help of thin layer chromatography and phytochemical screening. The chloroform and diethyl ether extracts of fresh, naturally and commercially dried *Z. officinale* revealed a significant antibacterial activity against *S. pyogenes, S. epidermidis*, *and S. aureus* by agar disc diffusion method while low activity was observed against *P. aeruginosa* and *E. coli.* As both chloroform and diethyl ether are non-polar solvents so we can say that there may be some non-polar compounds in *Z. officinale* having significant antibacterial activity.

The significant antibacterial activity of *Z. officinale* was indicated through TLC-bioautography especially against *S. aureus* as described by El-baroty *et al*. (2010). For *Z. officinale*, the most active compounds which induced clear inhibited zones on TLC plate had *Rf* values of 0.56, 0.43 and 0.37 correspond to cinnamyl aldehyde, eugenol, and methyleugenol as it was determined through a literature survey. Thus, these observations emphasize that these main compounds were mainly the contributor to the antibacterial property of *Z. officinale*. The significant antibacterial activity of *Z. officinale* is due to the presence of phytochemicals, which are bioactive, non-nutrient, naturally occurring plant compounds found in vegetables, fruits and spices (Okarter *et al*., 2009). *Z. officinale* extracts had dependent anti-microbial activity in the form dose against some bacteria and fungus such as *Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Salmonella typhimurium* (Jagetia *et al*., 2003). Indu *et al*. (2006) reported that the *Z. officinale* extract possessed effective antibacterial properties against *B. subtilis* and *E. coli.* The antibacterial efficacy of *Z. officinale* was also confirmed by Adonizio *et al.* (2006). Earlier studies indicated that ethanolic and methanolic *Z. officinale* extracts contain potent antimicrobial property against four strains of pathogenic bacteria through disc diffusion method (Sasidharan and Menon, 2010) and well diffusion method (Alagesaboopathi, 2011). These bacterial pathogens were (*E. coli, E. faecalis, P. aeruginosa, S. aureu*). In the present study, almost all *Z. officinale* extracts showed significant antibacterial activity due to the occurrence of certain secondary metabolites bioactive compounds, and phytochemicals. Our results are also as good as with the previous several studies (Ali *et al*., 2005). In general, we may conclude that the presence of these phytochemicals could account for much-touted medicinal properties of these spices in various disease conditions such as atherosclerosis, asthma, worm expeller, bacterial infections and cancer (Kaur and Arora,2009).

Antioxidant activity of A. sativum and Z. officinale

Pharmacological effects of most natural products used for medicinal purposes have been correlated to their possession of antioxidant activity (Sofidiya *et al*., 2006). Antioxidant capacity is the ability to reduce oxidative reactions within the human body. It is mainly due to their redox properties, which play a significant role in neutralizing and absorbing free radicals or decomposing peroxides (Louli *et al*., 2004). Antioxidant activity of *A. sativum* and *Z. officinale* has been attributed to the phenolic, organosulphur compounds, flavonoids and catalytic action of catalase and glucose oxidase (Djeridane *et al*., 2006). The high antioxidant activity of *A. sativum* was reported by numerous investigators (Miller *et al*., 2000). A lot of work has been done on the antioxidant activity of various solvent extracts of *A. sativum*. Sultana *et al.,* (2010) worked on a methanolic crude extract of *A. sativum* and *Z. officinale* by DPPH scavenging method with IC_{50} values of 89.25 48 µg/ml. Many researchers studied the overall antioxidant activity of fresh and dehydrated *A. sativum* (Gnanadesigan *et al*., 2011). Chung, (2006) worked on chloroform and ethanolic extracts of *A. sativum* and reported that they had high antioxidant activity.

Wangcharoen and Morasuk, (2009) also worked on antioxidant activity of dry and well-cooked *A. sativum*. They found that heating caused a reduction in antioxidant activity due to decomposition of phenolic and S-containing compounds. They also found that $ABTS^+$ assay was a better method for expressing antioxidant activity. The reported data showed the amount of bioactive compounds and their antioxidant activities in the studied raw *A. sativum* and *Z. officinale* to be greatly variable, depending on the extraction procedure (Pellegrini *et al*., 2007). Chen *et al.* (2008) reported highest (80%) antioxidant potential of both methanolic and ethanolic *Z. officinale* extracts. Methanolic extracts of *Z. officinale* have superior free radical scavenging ability and can be used as a primary antioxidant (Kikuzaki and Nakatani 1993). Morakinyo was reported the 93.9% and 95.1% $ABTS^+$ scavenging activities of the both ethanolic and aqueous extracts of *Z. officinale* (Morakinyo *et al*., 2012).

Metal chelating of A. sativum and Z. officinale extracts

Three different aqueous extracts of *A. sativum* and *Z. officinale* (fresh, naturally dried and commercially available) were tested for metal chelating potential. Results showed that all extracts of *A. sativum* and *Z. officinale* showed stronger metal chelation potential. Comparison of effect of incubation time of metal ion chelating potential of the extracts revealed that after incubation metal chelating potential of all *A. sativum* extracts was considerably increased. On the other hand metal chelating potential of all *Z. officinale* extracts was considerably decreased after incubation. While the extract CDZ showed same behavior after incubation**.** There are positive relationships between presence of metal chelating activity and phenolic compounds, based on the findings of several previous studies (Silva *et al*., 2007). Most of the researches have mentioned that high phenolic content will lead to high metal chelating activity. The present study on *A. sativum* and *Z. officinale* showed a positive relationship between results of phytochemical assay and metal chelating activity. *A. sativum* and *Z. officinale* are proven to be better metal chelators and they expressed high phenolic content. *A. sativum* possesses significant metal chelating activity maximum 45.00±1.73% at the highest sample concentration of 1.0 mg/mL (Siti *et al*., 2011). Chung-Yi *et al*., (2012) reported <10.00% metal chelating activity of *Z. officinale* while our extracts showed activity of 45-90% which may be due to difference in extraction procedure or incubation time.

Phytochemical screening and bioautograpghy

Many medicinal plants contain large amount of antioxidants such as flavonoids, polyphenols etc. Previous studies have shown that some flavonoids components such as rutin had anticancer activities and was able to inhibit cancer cell growth (Elattar and Virji, 2000). Gallic acid was reported as a free radical scavenger and as an inducer of differentiation and apoptosis in several types of cancer (lung cancer, leukemia, and colon adenocarcinoma cell lines (Sohi *et al*., 2003). Hence, the results of this research showed that flavonoids, phenols and other phytochemicals are important components of this plant, and some of their pharmacological effects could be attributed to the presence of these valuable constituents. Our results are similar to that reported by Sun and Ho, (2005) where chloroform/ methanol solvent system was comparatively more effective in extracting phenolic components from oat bran and other plant extracts. In that study, the content of phenolic components extracted by chloroform/ methanol system moved higher than that extracted by acetone. Turkmen *et al.* (2006) reported that solvent of different polarity had significant effect on polyphenol. Our results are consistent with the work of Turkmen *et al.* (2006). These results also revealed that methanolic solvent system is much better for separation of components than other less polar or non polar systems. Research conducted by Jayaprakash *et al*. (2001) confirmed the ineffectiveness of acetone, methanol and water for the extraction of total phenols from grapes seeds (*Vitis vinifera*). However, ethanol/ water or acetone/ water were better solvents compared to ethanol or acetone for the separation of plants components (Yilmaz *et al*., 2006). Our results have reasonable similarities with some earlier reports stating that non polar compounds like hydrocarbons, fatty acids and waxes present in natural products could be extracted with chloroform/ methanol solvent system (Stalikas, 2007).

When we discuss about the different spot colors, we find interesting literature about the color tests for identification of different colors of various compounds. As in the present study, the ethanolic and methanolic extracts of F.G and N.D.G showed red to orange band when stained with anisaldehyde/ sulphuric acid. It was reported in the literature that these specific spots indicate the presence of phenols in the extracts being studied (Stahl, 1969). On the other hand, diethyl ether and chloroform extracts of F.G and N.D.G expressed violet and purple color spots on staining with the same reagents in the present study. From the previous literature, it was observed that on staining with this reagent, the above mentioned colors indicate the presence of hydroquinone and other hydroxypheny-lpropane derivatives (Yu *et al*., 1994). Ethanolic extracts of N.D.G, F.G, chloroform, ethanolic and methanolic extracts of F.Z and N.D.Z showed brownish color indicating the presence of terpenes. Solvent extracts of F.G, N.D.G, F.Z and N.D.Z give pink to brown and yellow color under the UV light (365 nm) these are alkaloids (Yu *et al*., 1994). All these compounds were already reported in the present study through phytochemical analysis of these extracts and TLC results complemented the same. The components of *A. sativum* can be successfully separated using TLC method. Kanaki and Rajani, (2005) reported that for the separation of various compounds present in *A. sativum*, TLC method was found to be more specific, accurate and precise.

Separation of constituents of *Z. officinale via* TLC method has also been reported in earlier studies (Kikuzaki *et al*., 1999).

FTIR spectroscopy

In our results *A. sativum* have strong IR stretching vibrations in FT-IR spectroscopy as compared to *Z. officinale*. According to our results *A. sativum* have highly polar compounds while *Z. officinale* possessed mostly non polar active compounds as it was also confirmed by the results of antimicrobial and antioxidant activities of the current studies. In both of these assays polar extracts of *A. sativum* showed much better activities as compared to non polars extracts, and the case is reverse for *Z. officinale* extracts. So, the polar groups such as C=O, N-H, and O-H have strong IR stretching vibrations in FT-IR spectroscopy, and non polar groups such as C-C and S-S don't have intense bands in FT-IR spectroscopy. The bands at various wave numbers cm^{-1} are consistent with the previous literature (Movasaghi *et al*., 2008).

CONCLUSION

The present study was planned to explore antibacterial, antioxidant, metal chelating potential, phytochemical analysis, TLC thin layer bioautography and FT-IR Spectroscopy of secondary metabolites present in various natural and commercial extracts of *A. sativum* and *Z. officinale* either alone or in combination. The high antimicrobial and antioxidant effects of different tested extracts validated their therapeutic use. Similarly, phytochemical analysis and TLC studies of the same extracts of plants showed the presence of bioactive compounds responsible for medically important activities. So, we conclude that the extracts of these medicinal plants can be effectively used as a potential antimicrobial, antioxidant and metal chelators agents to overcome the problem of bacterial infections and multidrug resistant microbial strains, as to enable and enhance the market revenue throughout the world. The present study has provided some comparative biochemical information on the phytochemistry of *A. sativum* and *Z. officinale* found in AJK. There are indications that both plants are good source of nutrients and phytochemicals, so their use as nutritional supplements is highly promising.

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