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Antibacterial and Antioxidant Activities of Traditional Herbs and Honey against Fish Associated Bacterial Pathogens

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Abstract.- Antibacterial activity of four traditionally used medicinal plants *viz., Zingiber officinale, Allium sativum, Mentha spicata* and *Ocimum basilicum* have been studied against fish bacterial pathogens, using agar disc diffusion method. Bacterial pathogens including *Shigella flexneri, Enterobacter amnigenus, Salmonella typhimurium,* and *Serratia odorifera* were isolated from spoiled *Rita rita* and *Schizothorax plagiostomus.* Ethanolic and methanolic extracts of naturally dried *A. sativum* and *Z. officinale* significantly inhibited the growth of *S. typhimurium, S. flexneri* and *E. amnigenus*. Additionally, all extracts of *O. basilicum* substantiated moderate inhibition of all tested pathogens whereas ethanolic, diethyl ether and chloroform extracts of *M. spicata* corroborated low effect against all pathogens except *S. flexneri*. Honey also inhibited growth of *S. odorifera, E. amnigenus,* and *S. flexneri*. Activity index analysis showed the potential use of these plants and honey against *S. flexneri* when compared to antibiotics. All extracts of medicinal plants also indicated the significant antioxidant activity. The present study shows that these traditional medicinal plants and honey could potentially be used as antibacterial and antioxidant agents.

Keywords: Antibacterial activity, food borne pathogens, medicinal plants, honey, synergistic effect.

INTRODUCTION

Human infections are commonly due to bacterial pathogens, some of which are being transmitted from fish kept for both food and hobby. Various bacterial pathogens associated with human infections have been isolated from fish such as *Streptococcus, Escherichia coli, Salmonella*, and *Staphylococcus* (Novotny *et al*., 2004). Antimicrobial agents such as phytochemical products and antibiotics are being employed for the treatment of microbial infections. However it has been noted that a higher risk of microbial resistance exist in a region where the use of antibiotics both in human and pets have not been adequately controlled such as Pakistan where there is no legislation governing the use of antibiotics (Abraham, 2011). Medicinal plants would be the superlative source to attain a variety of drugs according to World Health Organization (WHO) because multidrug resistance microbial pathogens have been a great concern worldwide (Santos *et al*., 1995). The use of plant extracts and their phytochemicals having antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Awan *et al*., 2013; Ume-Kalsoom *et al*., 2013; Toroğlu and Çenet, 2013: Nasim *et al*., 2012; Daka, 2011; Shekhawat and Vijayvergia, 2010; Shokradeh and Ebadi, 2006).

Both *Mentha spicata* and *Ocimum basilicum*, are members of *Lamiaceae* family whereas *Allium sativum* and *Zingiber officinale* belong to *Alliaceae* and *Zingiberaceae* families, respectively. These are annual herbs, which grow in several regions around the world. The fresh and dried *M. spicata* plant is used in toothpaste, food, confectionary, cosmetic, pharmaceutical industries, and chewing gum (Znini *et al*., 2011; Lawrence, 2006) whereas *O. basilicum* is used as an antibacterial against poisoning microbes of food products (Politeo *et al*., 2007). Historically, *A. sativum* and *Z. officinale* have been used for centuries throughout the world by various 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; Mahmoodi *et al*., 2006). So far the effect of *M. spicata* and *O. basilicum* on fish associated pathogens along with natural honey has not been investigated.

Current research hypothesized that a possible

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synergy of *M. spicata*, *O. basilicum A. sativum*, *Z. officinale* along with honey could result in a reduction in the use of antibiotics. In this study antibacterial effect of *M. spicata*, *O. basilicum*, *A. sativum* and *Z. officinale* extracts and honey have been assessed against fish associated pathogenic bacteria. Antioxidant activity of these medicinal plants and honey was also evaluated through $ABTS^{\cdot+}$ {2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)} free radical scavenging activity.

MATERIALS AND METHODS

Sampling

Fresh leaves of basil (*Ocimum basilicum*) and mint (*Mentha spicata*), and bulb of garlic (*Allium sativum*) were collected from different localities of Muzaffarabad, whereas rhizome of ginger (*Zingiber officinale*) was purchased from the supermarket, Muzaffarabad, Azad Jammu and Kahsmir, Pakistan. Medicinal plants were identified by a taxonomist at the Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. Samples of all medicinal plants were thoroughly rinsed with running tap water to remove sand and other debris. Rinsed plant materials were air dried under shade for complete drying and preserved at room temperature. Natural honey of local eucalyptus plants was collected from Kail region of Neelum Valley, Azad Kashmir, Pakistan.

Extract preparation

Solvent extracts of medicinal plants were prepared through conventional solvent extraction methods. Dried leaves of *M. spicata* and *O. basilicum*, bulb of *A. sativum* and rhizome of *Z. officinale* were crushed, 30 and 20 mg of crushed leaves were soaked separately in 200 ml of organic solvents in increasing order of polarity (Diethyl ether, chloroform, ethanol and methanol) for 15-25 days. Each extract was filtered by Whatman No.1 filter paper, concentrated and stored at room temperature for further processing (Nasim *et al*., 2012). Different concentrations of honey *viz*., 1%, 5%, 10%, 15% and 20% (w/v) were prepared with double distilled water. This was done by dissolving 20 g of crude honey in 100 ml of double distilled water and filtered. Further concentrations were prepared through serial dilutions of 20% honey. For synergy the equal volume of each fresh extract and honey (500 µl) was mixed together as 1:1 ratio and left for 24 h at room temperature.

Test microorganisms

For current research work, the fish associated bacterial pathogens *viz*., *Shigella flexneri*, *Enterobacter amnigenus*, *Salmonella typhimurium*, and *Serratia odorifera* were taken from Biotechnology Laboratory, Department of Zoology, Azad Jammu and Kashmir University, Muzaffarabad, Pakistan (Ume-Kalsoom *et al*., 2013).

Antibacterial activity

By agar disks diffusion method the antibacterial activity of all solvent extracts and honey was tested against bacteria isolated from fish (Martinez-Vazquez *et al.*, 1999). The microorganisms were activated by inoculating a loop full of strain in 25 ml of nutrient broth medium (NBM; Oxoid CM001) 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 (NAM; Oxoid CM1) at 45°C and poured the sterilized plates. All the plates were placed at room temperature in laminar flow to solidify. The discs of 5 mm were prepared as follows, soaked with 200 µl of a particular extract and honey or the corresponding solvent was applied on discs 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 negative control. The inhibition of tested bacteria was measured by determining the diameter of the zone of inhibition after 24-48 h in millimeter (Seeley *et al*., 2001). The results of the sensitivity tests were expressed as (0) for no sensitivity, + (below 4 mm) for low sensitivity, ++ (4-8 mm) for moderate sensitivity and +++ (9-19 mm) for high sensitivity.

Sensitivity test

Sensitivity of antibiotics (positive control) such as ampicillin and trimethobrim against test

microbial strains was assessed through agar disc diffusion method (Bauer *et al*., 1966). Sensitivity was predicted with degree of clear zone surrounding the disc.

Determination of antioxidant activity

The chemical reagent ABTS {2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid} was used to evaluate the antioxidant potential of extracts of medicinal plants, and honey as described by 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 minimum 16 h to generate $ABTS^+$ free radicals. On the other hand, the running solution was organized by diluting 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 10 µl extracts of different solvents (0-100 µg/ml). The absorbance of test samples (Ai_{Sample}) was also observed at 734 nm exactly 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 calculated using the formula:

$$\% \text{ RSC} = [(Ao_{Control} - Ai_{Sample}) / Ao_{Control}] \text{ x}100\%.$$

Statistical analysis

Each experiment was repeated in triplicates and Mean ± Standard Deviation from absolute data was calculated. The comparison of antibacterial activity of solvent extracts of medicinal plants and different concentrations of honey with standard antibiotics was also determined through activity index (AI) (Shekhawat and Vijayvergia, 2010). To establish activity index following formula was used {Activity index= zone of inhibition of extract / zone of inhibition of antibiotic}.

RESULTS AND DISCUSSION

The antibacterial activity of naturally dried medicinal plants has been elucidated against fish associated bacterial pathogens *viz*., *S. flexneri, E. amnigenus, S. typhimurium*, and *S. odorifera* through agar disc diffusion method. Results found in this study were similar to those obtained by Bakht *et al*. (2011) where the inhibition of tested bacteria was illustrated in varying degrees by plant extracts.

Antibacterial activity of medicinal plants

The antibacterial activity of *A. sativum* against bacterial pathogens including Gram positive and Gram negative bacteria had been reported (Durairaj *et al*., 2009; Eja *et al.,* 2007). It had been observed that ethanolic and methanolic extracts of naturally dried *A. sativum* showed significant inhibition of *S. flexneri* (16.00±6.00 mm and 17.00 ±1.40 mm). Similarly, ethanol extract also showed the significant inhibition of *E. amnigenus* (12.00±2.00 mm) whereas moderate effect was recorded for methanol extract (10.00±0.47 mm). However, low sensitivity of extracts was measured against *S. typhimurium* (Table I). The antimicrobial activity shown by *A. sativum* extracts in this study agrees with the findings of others (Bakht *et al*., 2011; Sebiomo *et al*., 2011; Belguith *et al*., 2010; Sadeghian and Ghazvini, 2002). In some studies aqueous extracts of *A. sativum* has found to be more potent than the organic extracts (Roy *et al.*, 2006; Jaber and Al-Mossawi, 2007).

It had been observed that *E. amnigenus* and *S. typhimurium* were sensitive to methanolic extracts of naturally dried *Z. officinale* (19.00±0.81 mm, 15.00±2.30 mm). Similarly, ethanol extract showed maximum inhibition of *E. amnigenus* (12.00±1.40 mm; Table I). *S. flexneri* was resistant to diethyl ether extract of naturally dried *Z. officinale.* These findings are quite consistent with those elucidated by Senhaji *et al.* (2007) and Akoachere *et al*. (2002). Methanolic extract of *M. spicata* showed moderate inhibition of *S. flexneri* (4.33±2.08 mm) whereas ethanolic, diethyl ether and chloroform extracts had low effect (3.33±1.52 mm, 0.66±0.58 mm and 2.00±0.00 mm; Table I). *S. typhimurium* was slightly sensitive towards all extracts of *M. spicata* whereas substantiated moderate sensitivity was observed towards methanolic and ethanolic extracts of *O. basilicum* (4.00±1.00 mm and 7.33±2.51 mm), respectively.

On the contrary, all extracts of *O. basilicum* substantiated moderate inhibition of *S. flexneri* (7.33±5.80 mm, 5.33±2.51 mm, 4.66±1.15 mm, and 6.33±0.58 mm; Table I). *S. odorifera* exhibited moderate sensitivity towards diethyl ether extract of

Table I.- Effect of different extracts of medicinal plants (*Ocimum basilicum, Mentha spicata, Allium sativum, Zingiber officinale*) on the growth of various bacterial pathogens.

Medicinal plants	Extracts	Zone of inhibition in mm (M ± SD)			
		S. typhimurium	*S. flexneri*	*S. odorifera*	*E. amnigenus*
Mentha spicata	Diethyl ether	1.33±0.58*	0.66±0.58*	4.66±0.58**	1.66±0.58*
	Chloroform	1.33±0.58*	2.00±0.00*	1.33±0.58*	2.00±0.00*
	Ethanol	1.33±0.58*	3.33±1.52*	1.33±0.58*	3.66±0.58*
	Methanol	3.00±1.00*	4.33±2.08**	2.00±1.00*	2.66±2.08*
Ocimum basilicum	Diethyl ether	2.66±0.58*	7.33±5.80**	1.00±0.00*	3.00±2.00*
	Chloroform	2.66±0.58*	5.33±2.51**	3.33±1.52*	3.33±1.15*
	Ethanol	4.00±1.00**	4.66±1.15**	1.00±0.00*	3.66±1.52*
	Methanol	7.33±2.51**	6.33±0.58**	1.00±0.00*	3.33±2.08*
Allium sativum	Diethyl ether	5.00±2.00**	7.00±2.00**	5.00±0.94**	4.00±0.47**
	Chloroform	1.00±0.00*	6.00±0.47**	3.00±0.47*	2.00±0.47*
	Ethanol	3.00±0.47*	16.00±6.00***	8.00±2.00**	12.00±2.00***
	Methanol	3.00±0.47*	17.00±1.40***	6.00±1.20**	10.00±0.47***
Zingiber officinale	Diethyl ether	5.00±0.94**	0.00±0.00	2.00±0.81*	3.00±0.47*
	Chloroform	6.00±1.40**	2.00±0.99*	4.00±1.24**	4.00±1.24**
	Ethanol	8.00±0.47**	7.00±1.24**	9.00±2.40***	19.00±0.81***
	Methanol	15.00±2.30***	8.00±1.40**	7.00±1.24**	12.00±1.40***

Growth of inhibition was expressed as (0) for no sensitivity, * (< 4 mm) for low sensitivity, ** (>4-8 mm) for moderate sensitivity and *** (>8-19 mm) for high sensitivity. **M±SD**; indicates Mean ± Standard deviation.

M. spicata with 4.66±0.58 mm zone of inhibition whereas all extracts of *O. basilicum* showed low inhibition of *E. amnigenus* (Table I). Our results are in agreement with those of Adeola *et al.* (2012), Bharathi *et al.* (2011) and Sokovic *et al.* (2007). It was observed that all control solvents had no effect on all tested bacterial pathogenic.

Antibacterial activity of natural honey

In current research minimum inhibitory concentration of natural honey was evaluated and it was observed that *S. odorifera* was significantly inhibited by 10%, 15%, and 20% (10.00±0.81 mm, 10.60±1.24 mm, and 11.30±1.24 mm), *E. amnigenus* by 10% (8.00±2.16 mm), and *S. flexneri* by 10% and 15% (10.00±2.4 mm and 10.00±0.81 mm; Table II). On the other hand low sensitivity was recorded by 1% and 5% against all tested pathogens. Our results are consistent with previous studies in which significant effect of honey against tested pathogens was found (Alnaimat *et al.*, 2012; Halawani and Mohammed, 2011; Mandal and Shyamapada, 2011; Voidarou *et al.*, 2011; Maddocks *et al.*, 2012).

Combined antibacterial effect of medicinal plants and honey

The maximum inhibition of *S. odorifera* (F) was recorded when different concentrations of honey was used with *Z. officinale* as 1% (8.30±0.47 mm), 5% (8.30±1.24 mm), and 20% (9.60±1.24 mm; Table II). On the other hand *S. typhimurium* was moderately inhibited while the greatest inhibition of *E. amnigenus* was also recorded when honey was used with *Z. officinale* as 10% (10.00±1.60 mm) and 20% (9.60±1.24 mm). Similarly, *S. flexneri* was significantly inhibited by *M. spicata* and honey such as 10% and 15% (10.33±1.52 mm, and 9.66±2.51 mm) whereas moderately inhibited when *O. basilicum* was used with honey (Table II).

Activity index (AI) analysis against pathogens

Sensitivity test of trimethobrim and ampicillin were tested against all tested fish bacterial pathogenic (Table III). Sensitivity test

Table II.- Synergy of honey concentrations with medicinal plants.

1:1 ratio of extracts of medicinal plants with honey concentration	Honey concentration	Zone of inhibition in mm (M ± SD)			
		S. typhimurium	*S. flexneri*	*S. odorifera*	*E. amnigenus*
Honey	1%	3.00±0.81*	4.30±0.47**	2.60±0.47*	2.60±0.47*
	5%	5.00±0.81**	7.60±0.47**	3.00±0.81*	3.00±0.81*
	10%	4.30±0.47**	10.00±2.40***	10.00±0.81***	8.00±2.16**
	15%	3.00±0.81*	10.00±0.81***	10.60±1.24***	2.00±0.81*
	20%	3.00±1.40*	2.30±0.94*	11.30±1.24***	2.60±0.47*
A. sativum + honey	1%	6.00±0.81**	0.00±0.00	6.60±2.00**	2.60±0.47*
	5%	4.00±2.16**	0.00±0.00	4.00±0.81**	2.30±0.47*
	10%	4.30±2.05**	5.30±1.24**	4.30±0.47**	3.60±0.47*
	15%	1.00±0.00*	3.00±0.81*	6.30±2.00**	3.60±0.94*
	20%	7.00±2.16**	2.60±1.7*	4.30±0.94**	5.30±1.24**
Z. officinale + honey	1%	4.30±3.30**	1.30±0.47*	8.30±0.47***	2.30±0.47*
	5%	6.60±0.47**	4.00±2.16**	8.30±1.24***	2.60±0.47*
	10%	6.60±0.47**	2.00±0.00*	4.30±1.24**	10.00±1.60***
	15%	5.30±2.05**	2.00±0.81*	5.60±1.24**	9.00±0.81***
	20%	4.60±2.05**	5.00±2.40**	9.60±1.24***	9.60±1.24***
M. spicata + honey	1%	1.66±0.57*	3.66±0.57*	4.66±0.57**	1.66±0.57*
	5%	1.66±0.57*	4.00±0.00**	1.33±0.57*	2.00±0.00*
	10%	2.33±0.57*	10.33±1.52***	1.33±0.57*	3.66±0.57*
	15%	1.66±1.00*	9.66±2.51***	2.00±1.00*	2.66±2.08*
	20%	1.00±0.00*	3.66±0.57*	3.66±1.52*	1.00±0.00*
O. basilicum + honey	1%	1.00±0.00*	7.33±5.80**	1.00±0.00*	3.00±2.00*
	5%	1. 33±0.57*	5.33±2.51**	3.33±1.52*	3.33±1.15*
	10%	1.66±0.57*	4.66±1.15**	1.00±0.00*	3.66±1.52*
	15%	1.33±0.57*	6.33±0.57**	1.00±0.00*	3.33±2.08*
	20%	2. 33±0.57*	7.66±0.57**	3.66±0.57*	7.33±2.08**

Growth of inhibition was expressed as (0) for no sensitivity, * (< 4 mm) for low sensitivity, ** (>4-8 mm) for moderate sensitivity and *** (>8-19 mm) for high sensitivity. **M±SD**; indicates Mean ± Standard Deviation

indicated that trimethobrim and ampicillin showed moderate inhibition of *S. typhimurium*. On the other hand trimethobrim showed the maximum inhibition of *S. odorifera* and *E. amnigenus* while ampicillin had no effect on these bacterial pathogens. Trimethobrim and ampicillin had no effect on the growth of *S. flexneri* (Table III). Activities index indicated that all the extracts of plants and honey showed maximum inhibition of *S. flexneri* when compared to trimethobrim and ampicillin (Table III).

Antioxidant activity

The greatest antioxidant activity has been observed in polar extracts of *M. spicata* (94%, 91%, 87% and 81%), non-polar extracts of *O. basilicum* and naturally dried *Z. officinale,* and all extracts of *A. sativum* except diethyl ether (Fig. 1A). Our results are consistent with the work of Turkmen *et*

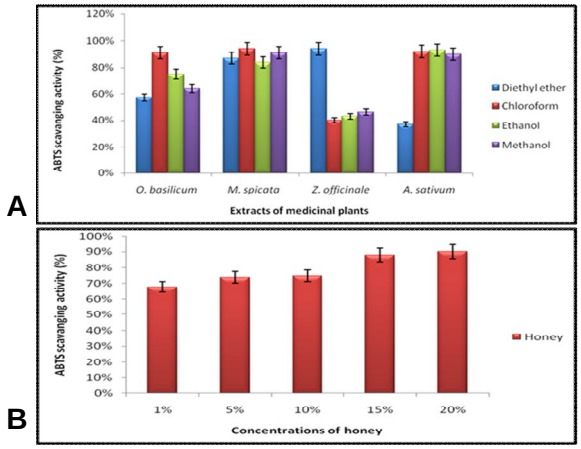

Fig. 1. Assessment of antioxidant potential of medicinal plants and honey concentrations through $ABTS^{\cdot+}$ free radical scavenging activity method. **A,** antioxidant activity of medicinal plants, **B,** antioxidant activity of natural honey.

Table III.- Activity Index of honey with medicinal plants.

Medicinal plants + honey	Bacterial pathogens	Activity index= zone of inhibition of extract / zone of inhibition of antibiotic					Antibiotics (Zone of inhibition mm)
Natural honey	*S. typhimurium*	0.42	0.71	0.61	0.42	0.42	Trimethobrim (7)
		0.42	0.71	0.61	0.42	0.42	Ampicillin (7)
	S. flexneri	E>A	E>A	E>A	E.A	E>A	Trimethobrim (0)
		E>A	E>A	E>A	E>A	E>A	Ampicillin (0)
	S. odorifera	0.32	0.37	1.25	1.32	1.41	Trimethobrim (8)
		E>A	E>A	E>A	E>A	E>A	Ampicillin (0)
	E. amnigenus	0.23	0.27	0.72	0.18	0.23	Trimethobrim (11)
		E>A	E>A	E>A	E>A	E>A	Ampicillin (0)
A. sativum + honey	*S. typhimurium*	0.85	0.57	0.61	0.14	1.0	Trimethoprim (7)
		0.85	0.57	0.61	0.14	1.0	Ampicilin (7)
	S. flexneri	E>A	E>A	E>A	E>A	E>A	Trimethoprim(0)
		E>A	E>A	E>A	E>A	E>A	Ampicilin (0)
	S. odorifera	0.82	0.5	0.53	0.78	0.53	Trimethoprim (8)
		E>A	E>A	E>A	E>A	E>A	Amprcilin (0)
	E. amnigenus	0.23	0.20	0.32	0.32	0.48	Trimethoprim (11)
		E>A	E>A	E>A	E>A	E>A	Amprcilin (0)
Z. officinale + honey	*S. typhimurium*	0.61	0.94	0.94	0.75	0.65	Trimethoprim (7)
		0.61	0.94	0.94	0.75	0.65	Ampicilin (7)
	S. flexneri	E>A	E>A	E>A	E>A	E>A	Trimethoprim(0)
		E>A	E>A	E>A	E>A	E>A	Ampicilin (0)
	S. odorifera	1.03	1.03	0.53	0.7	1.2	Trimethoprim (8)
		E>A	E>A	E>A	E>A	E>A	Amprcilin (0)
	E. amnigenus	0.20	0.23	0.90	0.81	0.87	Trimethoprim (11)
		E>A	E>A	E>A	E>A	E>A	Amprcilin (0)
M. sapicata + honey	*S. typhimurium*	0.23	0.23	0.33	0.23	0.14	Trimethoprim (7)
		0.23	0.23	0.33	0.23	0.14	Ampicilin (7)
	S. flexneri	E>A	E>A	E>A	E>A	E>A	Trimethoprim(0)
		E>A	E>A	E>A	E>A	E>A	Ampicilin (0)
	S. odorifera	0.5	0.41	0.37	0.41	0.58	Trimethoprim (8)
		E>A	E>A	E>A	E>A	E>A	Ampicilin (0)
	E. amnigenus	0.33	0.12	0.36	0.18	0.09	Trimethoprim (11)
		E>A	E>A	E>A	E>A	E>A	Ampicilin (0)
O. basilicum + honey	*S. typhimurium*	0.14	0.19	0.23	0.19	0.33	Trimethoprim (7)
		0.14	0.19	0.23	0.19	0.33	Ampicilin (7)
	S. flexneri	E>A	E>A	E>A	E>A	E>A	Trimethoprim (0)
		E>A	E>A	E>A	E>A	E>A	Ampicilin (0)
	S. odorifera	0.33	0.08	0.08	1.0	1.0	Trimethoprim (8)
		E>A	E>A	E>A	E>A	E>A	Ampicilin (0)
	E. amnigenus	0.18	0.15	0.12	0.09	0.42	Trimethoprim (11)
		E>A	E>A	E>A	E>A	E>A	Amprcilin (0)

E > A & > 1 indicate extracts of medicinal plants has higher effect against bacterial pathogens compared to antibiotics; A>E & <1 indicate antibiotics has higher effect against bacterial pathogens compared to extracts of medicinal plants; 1 indicates both have equal effect.

al. (2006). $ABTS^{\cdot+}$ scavenging activities of both ethanolic and aqueous extracts of *Z. officinale* (93.9% and 95.1%) was also reported by Morakinyo *et al.* (2011). On the other hand antioxidant activity of honey (1%, 5%, 10%, 15% and 20%) was measured as 68%, 74%, 75%, 88%, and 90%,

respectively. It was observed that concentrated honey has more scavenging activity compared to diluted honey (Fig. 1B). Honey is also reported to scavenge 2,2-diphenylpicrylhydrazyl (DPPH) effectively (Omotayo *et al*., 2012).

CONCLUSIONS

It was concluded that the leaf, bulb, and rhizome extracts of medicinal plants have high antibacterial effect as an individual agent and also when combined with natural honey, may be due to the presence of antioxidants. Future studies can be based on this research to know the exact mechanism of inhibition and to develop new antimicrobial agents.

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