Phytochemical Screening, Antioxidant and Antibacterial Evaluations of Two Zingiberaceae Plants

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ABSTRACT

The crude ethanolic extract of two commonly zingiberaceae medicinal plants *Zingiber officinale* (ginger) and *Curcuma longa* (turmeric) were screened for their phytochemical content which revealed the presence of alkaloids, glycosides, terpenoids, flavonoids, tannins and saponins. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and iron chelating assays were used for assessment of antioxidative properties using propyl gallate and EDTA as standards, respectively. The antioxidant activity of ginger (*Zingiber officinale*) was stronger than turmeric (*Curcuma longa*) by using DPPH assay. The ethanolic extracts also exhibited antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella abony*, *Staphylococcus aureus* and *Bacillus subtilis*. Both of the two extracts showed highest antimicrobial activity against the bacterial strains than some of the reference antibiotics such as Ampicillin, Azithromycin and Ciprofloxacin at concentration (10µg/ml).

Key words: Crude ethanolic extract; *Curcuma longa*; *Zingiber officinale*; antibacterial; antioxidant; DPPH radical scavenging assay; iron chelating assay

INTRODUCTION

Medicinal plants are important source for the verification of pharmacological effects and can be natural composite sources that act as new anti- infectious agents. Herbs and spices are an important part of the human diet. They have been used for thousands of years to enhance the flavour, colour and aroma of food and they are also known for their medicinal value, which forms one of the oldest sciences. Yet it is only in recent years that modern science has started paying attention to the properties of spices. Although as natural substances spices and herbs are easily absorbed by our bodies and generally do not have any adverse effects.

The Zingiberaceae is a well-known plant family in Southeast Asia and many of its species are being used in traditional medicine. The two Zingiberaceous plants, *Curcuma longa* (Turmeric or Kurkum) and *Zingiber officinale* (Ginger), were investigated as they are perennial herbs widely cultivated in tropical regions of Asia, and have been commonly used as medicinal plants and spices. The rhizomes of these two plants possess diverse biological activities, for instance, antimicrobial [Hsiang et al (2014) and Pokhrel et al (2012)], antiulcer [Rafatullah et al (1990)] anti-inflammatory [Menon et al (2007) and Penna et al (2003)], cytotoxic and anti-tumor [Kunnumakkara et al (2007), Anuchapreeda et al (2008) and Kim et al (2008)].

The uses of natural antioxidants from plant extracts have experienced growing interest from some human health professionals and consumer's concern societies [Suhaj et al (2006)].

The interest naturally occurring antioxidant is focused more on edible plants, especially spices and herbs [Huda et al (2009)], because they are an excellent source of phenolic compounds (flavonoids, phenolic acid and alcohols, tocopherols, tocotrienols), ascorbic acid and carotenoids which have been reported to show good antioxidant activity [Zheng et al (2001)].

Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease [Valko et al(2007)] because they are capable of slowing or preventing the oxidation of other molecules. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols [Sies et al (1997)].

This work aimed to evaluate and compare the phytochemical constituents, antioxidant and antibacterial activities of crude ethanolic extracts from *Zingiber officinale* (Ginger) and *Curcuma longa* (Tumeric). The bioactive properties were evaluated against five bacterial strains.

MATERIAL AND METHODS

The rhizomes of *Curcuma longa* and *Zingiber officinale* were purchased from a local market in Omdurman, Sudan and authenticated at the Botany department of the University of Khartoum.

Preparation of extract:

In this study, 100 g powder of *C. longa* and *Z. officinale* rhizomes were macerated in 200 ml ethanol (95%) for 24 hours at room temperature with occasional shaking. Then ethanol extracts were filtered off through a Whatman No. 1 filter paper. This step was performed three times. Finally, extracts were concentrated under reduced pressure at 50°C through rotary vacuum evaporator. The concentrated extracts were collected in a Petri dish and air dried at room temperature and the yield of the extracts were calculated.

Preliminary phytochemical screening:

The ethanolic extract of *Curcuma longa* and *Zingiber officinale* were screened to determine the presence of the following metabolites through preliminary phytochemical screening such as alkaloids, glycosides, terpenoids and steroids, flavonoids, saponins and tannin by the following standard procedures described by [Debiyi et al (1978), Roopashree et al (2008) and Sofowora (1993)].

Alkaloids [Mayer's test]:

1.36 gm of mercuric chloride dissolved in 60 ml and 5 mg of potassium iodide were dissolved in 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100 ml using distilled water. To 1ml of acidic aqueous solution of samples few drops of the reagent was added. Formation of pale yellow precipitate showed the presence of alkaloids.

Flavonoids:

In a test tube containing 0.5 ml of alcoholic extract of the samples, 5 to 10 drops of diluted HCl and small amount of Mg metal were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown colour indicated the presence of flavonoids.

Glycosides:

A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

Steroids [Salkowski's test]:

About 100 mg of dried extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface is an indicative of the presence of steroidal ring.

Tannins:

[Lead acetate test]:

In a test tube containing about 5ml of an aqueous extract, a few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicated the presence of tannins.

FeCl3 test:

A 2ml filtrates [200 mg of plant material in 10 ml distilled water, filtered], and 2ml of $FeCl_3$ were mixed. A black precipitate indicates the presence of tannins.

Terpenoids:

2ml of chloroform and 1ml of conc. H_2SO_4 was added to 1mg of extract and observed for reddish brown colour that indicates the presence of terpenoid.

Saponins:

2ml of the aqueous extracts were placed in a test tube and 2 ml of distilled water were added and the tube was shaken vigorously. Formation of persistent foam indicates the presence of saponins.

TLC analysis:

Small amounts of *C. longa and Z .officinale* extracts were dissolved in ethanol and spotted on precoated silica gelF_{254} aluminum plate of size (10×20) using capillary tube, n-hexan: ethyl acetate (6:4- 7:3) was used as solvent system. Visualization of the separated bands was carried out under U.V. light (365 nm). The colours and R_f values were recorded [Mabry et al (1970)]. In this study the plate was sprayed with Ceric Sulphate solution.

Determination of %Curcumin by UV/VIS spectroscopic method:

0.1g of dried extract was dissolved in 25 ml of ethanol. This solution was filtered and volume made up to 100ml. Then 10 ml of above solution was taken in volumetric flask and again volume made up to 100ml with ethanol [Rajpal et al (2005)]. The absorbance was measured at 425nm using s UV spectrophotometer (model UV- 1800).
% Curcumin = a×100/A×L×W
Where a, = absorbance of sample at 425nm = 0.44
L = path length (1cm)
A = molar absorptivity of standard curcumin [Himesh et al (2011)].

Determination of in vitro antioxidant activity:
DPPH radical scavenging assay:

The DPPH radical scavenging was determined according to the method of Shimada et. al. (1992), with some modification. In 96-wells plate, 10µl of the test samples were allowed to react with 90µl 2,2-diphenyl-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as (300µM). The test sample was dissolved in dimethyl sulphoxide (DMSO) while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 715nm using Thermo Scientific Skanlt multiplate reader spectrometer (model UV- ST_2. The percentage inhibition values were calculated from the absorbance of the control ($\boldsymbol{A_c}$) which is DMSO and of the sample ($\boldsymbol{A_s}$) using Eq. (1). Propyl gallate was used as positive controls. The values are presented as the mean of triplicate analyses

$$\textbf{Inhibition}\ (\%) = \frac{(\boldsymbol{A_c - A_s})}{\boldsymbol{A_c}} \times \mathbf{100} \qquad (\mathbf{1})$$

Iron chelating activity assay:

The iron chelating ability was determined according to the modified method of Dinis et. al. (1994). The Fe^{+2} were monitored by measuring the formation of purple ferrous ion-ferrozin complex. 10µl of each extract stock solution (5mg/ml) was mixed with 30µl of 2mM $FeSO_4$. The reaction mixture was incubated for 5 min, before the addition of 60µl of 5mM ferrozine. The mixture was shaken and left at room temperature for 10min. the absorbance was measured at 562nm. DMSO was used as control. The iron chelating activities were calculated from the absorbance of the control (***A**c*) and of the sample (***A**s*) using Eq. (1) and expressed as Na_2EDTA equivalents (mg Na_2EDTA/g extract). The values are presented as the means of triplicate analyses.

Determination of in vitro antibacterial activity:

Cup-plate agar diffusion method:

The cup-plate agar diffusion method was adopted to assess the anti-bacterial activity of the prepared extracts [Kavanagh et al (1972)]. 0.6 ml of standardized bacterial stock suspensions (10^8-10^9) colony-forming units per ml was thoroughly mixed with 60ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of this plates 2cups, 10 mm in diameter, were cut using a sterile cork borer No.4 and the agar disk were removed. Alternate cups were filled with 0.1 ml (10mg/ml) of each extract using micro-titer pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 73^{O}C for 24 hours. Two replicate were carried out for each extract against each of the test organism. After incubation the diametersof growth inhibition zones were measured, averaged values were tabulated.

RESULTS AND DISCUSSION

The percentage yields of extracts were calculated and the phytochemical constituents of the plants are shown in [Table 1]. The highest yield of ethanolic extract was found in *Zingiber officinale* (8.77%) and the lowest in *Curcuma longa* (3.98%). Phytochemical screening was carried out on *C. longa* and *Z. officinale* rhizomes ethanolic extract which revealed the presence of alkaloids, saponins, flavonoids, terpenoids, steroids and tannins. Glycosides were found only in Z. *officinale*. The quantitative determination of %curcumin was found to be 26.19%.

Table1: Results of the phytochemical screening of the plant extracts

Plant constituents	***C. longa***	***Z .officinale***
Alkaloids	++	+++
Flavonoids	++	+++
Glycoside	*-ve*	++
Steroid	++	+++
Terpenoids	++	+++
Tannins	++	+++
Saponins	++	++

+++= *abundant* ++= *moderate* *-ve= not detected*

Thin Layer Chromatography of *C. longa* extracts was performed on precoated silica gelF_{254} aluminum plate of size (10×20). The components in two extracts were separated and detected under UV light at 365 nm and after spraying with ceric sulphate resulted in the R_f values as

indicated in [Table 2]. About 6 spots of different colours were observed in the chromatograms of each extract. Yellow and violet colours indicated to falvonoids and terpenoids, respectively [Mabry et al (1970)].
The red spot (R_f value 0.23) indicate to curcumin which is the major component in *C. longa* as Himesh et al (2011) reported. [Figure1(I&II)]

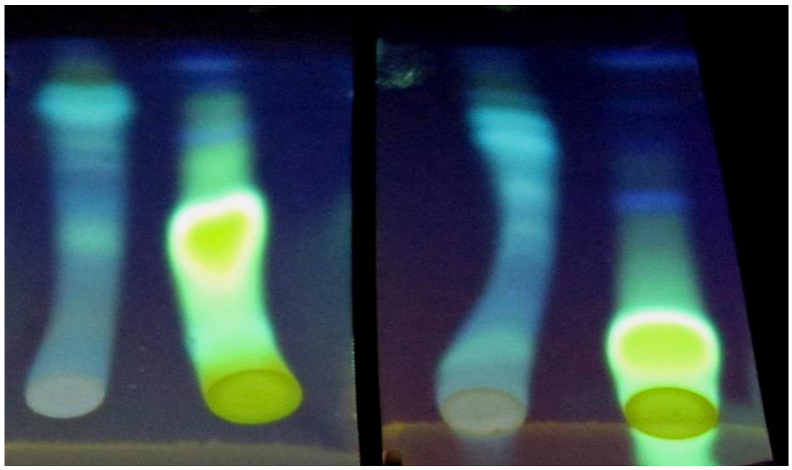
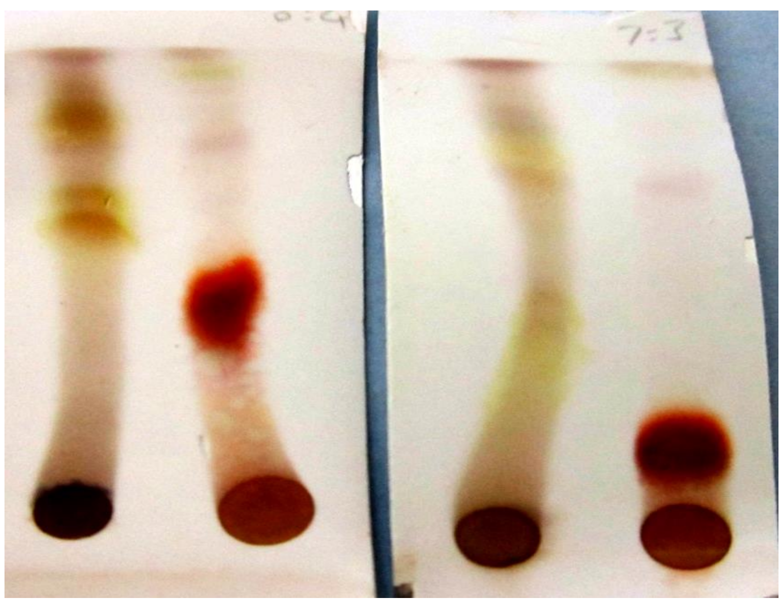


Figure1: I. TLC analysis of the C.longa (right) and Z.officinale (left) extracts detected under UV 365nm

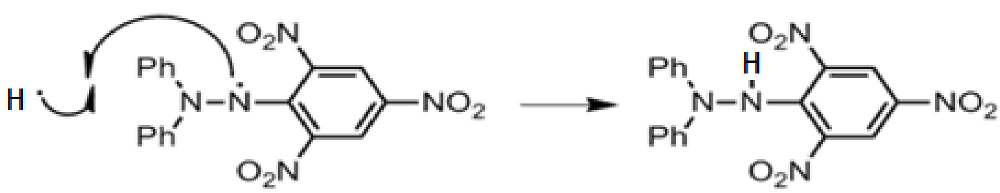


II. TLC analysis of the C.longa (right) and Z.officinale (left) extracts after sprayed with ceric sulphate

Table2: TLC profile of ethanolic extracts of C. longa and Z. officinale

Methods of detections					
C. longa			***Z. officinale***		
UV (365nm)	***Ceric sulphate***	***R_f values hex:eth (7:3)***	***UV (365nm)***	***Ceric sulphate***	***R_f values hex:eth (7:3)***
Yellow	Red	0.23	Blue-green	Yellow	0.34
Yellow-green	-	-	Blue-green	Yellow	0.50
Blue	Violet	0.68	Blue	Violet	0.68
Yellow-green	Pale-yellow	0.84	Blue - green	Yellow	0.75
-	-	-	Blue-green	Yellow	0.84
Blue	Violet	0.97	Blue	Violet	0.97

Plants have been reported to exhibit antioxidant activity due to the presence of antioxidant compounds such as phenolics, and flavonoids. DPPH radical was used as a stable free radical to determined antioxidant activity of natural compounds. The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals [Stoilova et al (2007)]. Thus, the purple colour of 2,2 diphenyl-1- picryl hydrazyl (DPPH) will reduce to α, α-diphenyl- β-picrylhydrazine (yellow colored) [Akowuah et al (2005)] Results of the activity of free radical scavenging of plants extracts are presented in [Table 3].



2,2 diphenyl-1- picryl hydrazyl (DPPH) ***α, α-diphenyl- β-picrylhydrazine***

Table3: In vitro antioxidant activity of ethanolic extract of C.longa and Z. officinale

Extract	**%RSA ± STD (DPPH)**	**% iron chelating ± STD**
C. longa	***74.5 ± 0.02***	***-ve***

Z. officinale	***88.7 ± 0.01***	***-ve***

Key (-ve): not active

Results showed that, Ginger extract contained the highest DPPH radical scavenging activity (88.7 ± 0.01%), followed by turmeric extract (74.5 ± 0.02%). This was in agreement with Maizura et al (2011) who reported that DPPH radical scavenging activity for ginger and turmeric were (79.0 ±0.6%) and (64.6 ± 2.4%) respectively, the lower values of inhibition may be due to the method of extraction which was juice extractor. The results obtained demonstrated that ginger had the highest phenolic content and antioxidant activity compared to turmeric. This finding is agreement with the phytochemical screening and TLC results.

The results of the DPPH free radical scavenging assay suggest that rhizomes of the two zingiberaceae plants have potent antioxidant property of scavenging free radicals. These species could be used as a potent source for the cancer chemo protective therapy.
In this study, the antioxidant activity is also determined on the basis of the ability of antioxidant in this plants extracts for ferrous (II) iron chelating. The transition metal ion, Fe^{2+} possesses the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$$

The main strategy to avoid reactive oxygen species (ROS) generation that is associated with redox active metal catalysis involves chelating of the metal ions. Hatchera et al (2008), Ahmed et al (2009) and Maizura et al (2011) were reported that the ginger and turmeric had iron chelating activity but in our study none of them revealed these activities and that may be due to uncontrolled pH condition of the reaction.

The antimicrobial activity of crude ethanolic extracts of *Z. officinale* and *C. longa* were tested on five bacterial strains, three Gram-negative *Escherichia coli* (E.c), *Pseudomonas aeruginosa* (Ps.a) and *Salmonella abony* (Sal)) and two Gram-positive *Staphylococcus aureus (S.a)* and *Bacillus subtilis* (B.s) [Figure2(I&II)]. The results of diameters of the zones of inhibition of extracts and antibiotics are presented in [Tables (4) and (5)] respectively, and were interpreted as sensitive (18mm), moderate (14-18mm) and resistant (<14mm) [Collins et al (1995)].

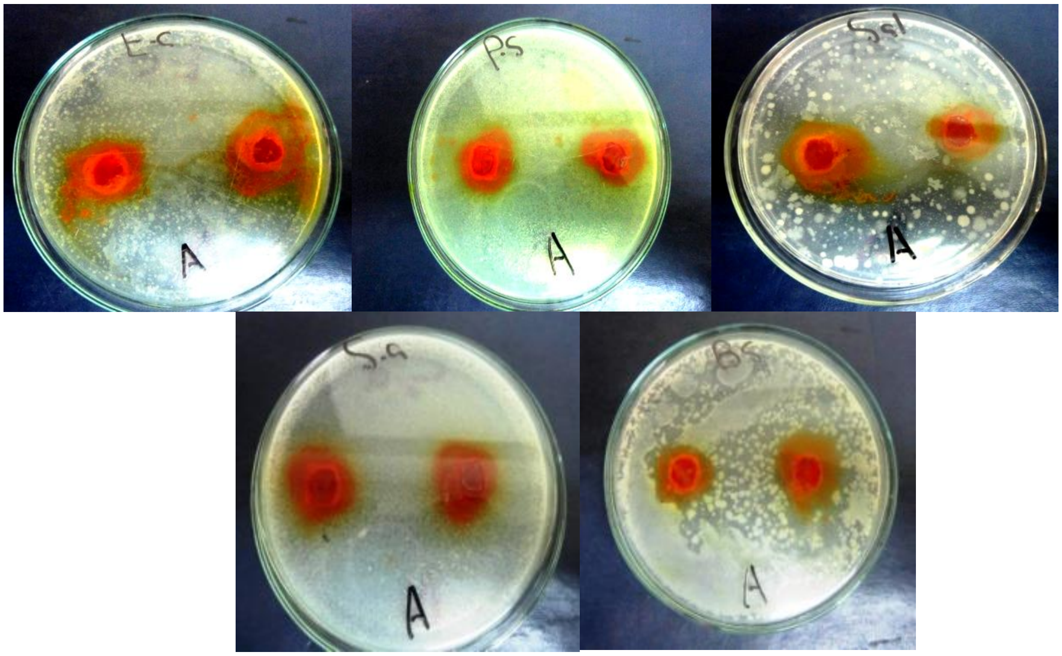


Figure2 :(I) Inhibition zone of five bacterial strains with turmeric extract

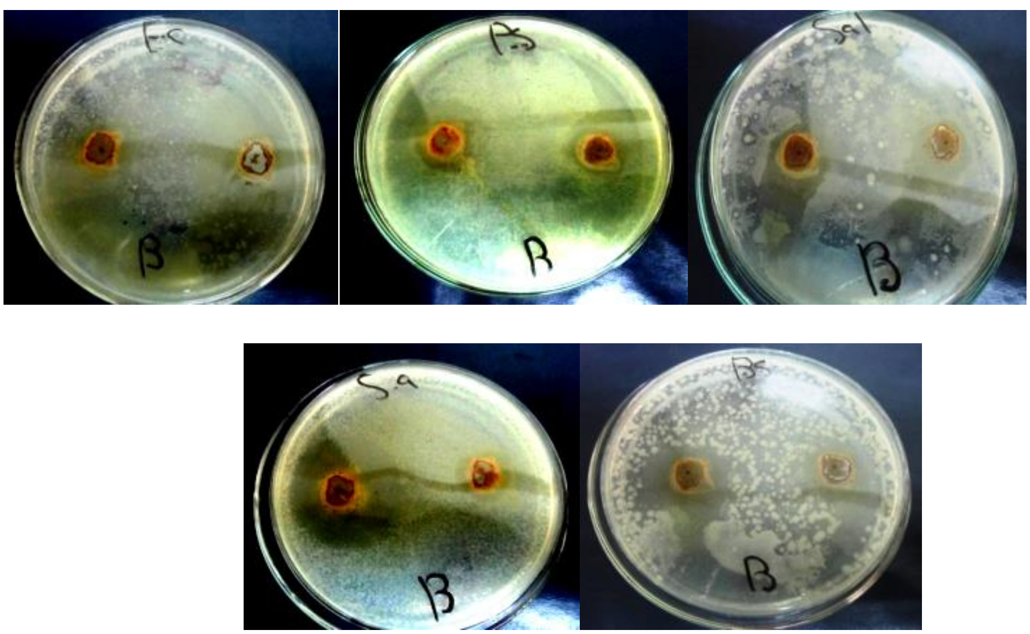


Figure2 :(II) Inhibition zone of five bacterial strains with ginger extract

Table4: zones of growth inhibition (mm) showing antibacterial activity for crude ethanolic plants extract (10µg/ml)

Plant extract	**Standard organisms/Mean diameter of inhibition zone(mm)**				
	E.c	**Ps.a**	**Sal**	**S.a**	**B.s**
C. longa	16	15	17	15	14.5
Z. officinale	19.5	17	18	18	19.5

Table5: antibacterial activity of reference drugs against standard organisms

Drugs	***Concentrations used(µg/ml)***	***Standard organisms/Mean diameter of growth inhibition zone (mm)***				
		E.c	***Ps.a***	***Sal***	***S.a***	***B.s***
Ampicillin	*40*	*17*	*20*	*25*	-	-
	20	*14*	*14*	*20*	-	-
	10	-	*14*	*14*	-	-
	5	-	-	-	-	-
Azithromycin	*40*	*15*	*20*	*24*	*24*	-
	20	*14*	*20*	*20*	*16*	
	10	*14*	*16*	*15*	-	
	5	-	*14*	*15*	-	
Ciprofloxacin	*40*	*15*	*20*	*25*	*20*	*20*
	20	*14*	*14*	*20*	*17*	*18*
	10	*14*	*14*	*20*	-	*14*
	5	-	-	*14*	-	-

The crude ethanolic extracts showed the broadest antibacterial activity by inhibiting growth of bacterial strains tested (the diameter of inhibition zone, 14.5-19.5mm) Ginger showed highest antimicrobial activity against all bacterial strains when it was compared to reference antibiotics than the turmeric at concentration (10µg/ml). Identical results were reported in ginger but via different methods as broth micro dilution method using three strains of pathogenic bacteria (Shipra et al 2012). Auta et al (2011) also reported antibacterial activity of ginger ethanolic extract on *E. coli* and

P. areuginosa. The highest activity of Ginger may be directly related to the most important pungent components of ginger (Cis-6-Shagole (7.45%), Gingerol (4.46%), Gingerol (1.98%) which have strong inhibitory activity against pathogenic bacteria [Park et al (2008)]. Curcumin is the most important fraction which is responsible for the biological activities of turmeric.

CONCLUSION

The ethanolic extracts obtained from rhizomes of *Curcuma long* (turmeric) and *Zingiber officinle* (ginger) have shown that *Zingiber officinale* had the highest phenolic content according to the phytochemical screening tests therefore had highest antioxidant and antibacterial activity compared to *Curcuma longa*. The results of the DPPH free radical scavenging assay suggest that rhizomes of the two zingiberaceae plants have potent antioxidant property of scavenging free radicals. These species could be used as a potent source for the cancer chemo protective therapy.

REFERENCES

Ahmed, S. H. (2009). Antioxidant properties of water extracts for the Iraqi plants Phoenix dactylifera, *Loranthus europeas*, *Zingiber officinalis* and *Citrus aurantifolia*. Modern Applied Science. 3(3): 161-166.

Akowuah, G. A., Ismail, Z., Norhayati, I. and Sadikun, A. (2005). The effects of different extraction solvents of varying polarities of polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. Food Chemistry. 93: 311-317.

Anuchapreeda, S., Tima, S., Duangrat, C. and Limtrakul, P. (2008). Effect of pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin on WT1 gene expression in leukemic cell lines. Cancer Chemother Pharmacol. 62: 585–594.

Auta, K. I., Galadima, A. A, Bassey, J. U., Olowoniyi, O. D., Moses, O. O. and Yako, A. B. (2011). Antimicrobial properties of the ethanolic extracts of *Zingiber officinale* (Ginger) on Escherichia coli and *Pseudomonas aeruginosa*. Annals of Biological Research. 2 (3): 307-311.

Bhargava, S., Dhabhai, K., Batra, A., Sharma, A. and Malhotra, B. (2012). *Zingiber officinale*: Chemical and phytochemical screening and evaluation of its antimicrobial activities. Journal of Chemical and Pharmaceutical Research. 4 (1): 360-364.

Collins, C. H., Lynes, P. M. and Grange, J. M. (1995). Microbiological Methods. pp 175-190 (7th Edn) Butterwort- Heinemann Ltd. Britain.

Debiyi, O. O. and Sofowora, F. A. (1978) Pytochemical screening of medical plants. loyidia. 3: 234-246.

Dinis, T. C. P., Maderia, V. M. C. and Almedia, L. M. (1994). Action of phenolic derivates (acetoaminophen, salyciate, and 5- aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys. 315: 161-169.

Hatchera, H., Planalpb, R., Chob, J., Tortia, F. M. and Tortic, S. V. (2008) Curcumin: From ancient medicine to current clinical trials. Cellular and Molecular Life Sciences. 9-10.

Himesh, S., Sharan, P. S., Mishra, K., Govind, N. and Inghai, A. K. (2011). Gualtitative and Quantitative profile of curcumin from ethanolic extract of *Curcuma longa*. International Research Journal of Pharmacy. 2 (4): 180-184.

Hsiang-yu,Y., Cheng-hung, C., Hsin-chun, C., Chu-jen, W., Tai-liang, C., Li-yun, L. (2014). Bioactive components analysis of two various gingers (*Zingiber officinale* Roscoe) and antioxidant effect of ginger extracts. LWT - Food Science and Technology. 55 (1):329- 334.

Huda, F. N., Noriham, A., Norrakiah, A. S. and Babji, A. S. (2009). Antioxidant activity of plants methanolic extracts containing phenolic compounds. African Journal of Biotechnology. 8 (3): 484-489.

Kavanagh, F., F.Kavanagh (Ed), (1972). Analytical Microbiology. Academic Press. 2: 11.

Kota, N., Krishna, P. and Polasa, K. (2008). Alterations in antioxidant status of rats following intake of ginger through diet. Food Chemistry. 106: 991- 996.

Kunnumakkara, A. B., Guha, S., Krishnan, S., Diagaradjane, P., Gelovani, J. and Aggarwal, B.B. (2007). Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-kappaB- regulated gene products. Cancer Res. 67: 3853–3861.

Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970). The systemic identification of flavonoids. springer-Verlag. New York.

Maizura, M., Aminah, A. and Wan Aida, W. M. (2011). Total phenolic content and antioxidant activity of kesum (*Polygonum minus*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) extract. International Food Research Journal. 18: 529-534.

Menon, V. P. and Sudheer, A. R. (2007). Antioxidant and anti-inflammatory properties of Curcumin. Advances in Experimental Medicine and Biology. 595: 105-125.

Park, M., Bae, J. and Lee, D. (2008). Antibacterial activity of 10-gingerol and 12-gingerol isolated from ginger rhizome against periodontal bacteria. Phytother Res. 22: 1446-1449.

Penna, S. C., Medeiros, M. V., Aimbire, F. S., Faria-Neto, H. C., Sertié, J. A. and Lopes-Martins, R. A. (2003). Anti-inflammatory effect of the hydralcoholic extracts of *Zingiber officinale* rhizomes on rat paws and skin edema. Phytomedicine. 10 (5): 381-385.

Pokhrel, S., Singh, R., Gautam, P., Dixit, V. K. and Das, A. J. (2012). Comparison of antimicrobial activity of crude ethanolic extracts and essential oils of spices against five strains of diarrhea causing *Escherichia coli*. Int. J. of Pharm. & Life Sci. (IJPLS). 3: 1624-1627.

Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992). Antioxidative properties of Xanthan on the antioxidation of soybean oil in in cyclodextrin emulsion. J Agric Food Chem. 40: 945-948.

Shipra, B., Kshipra, D., Amla, B., Asha, S. and Bharti, M. (2012). *Zingiber officinale*: Chemical and phytochemical screening and evaluation of its antimicrobial activities. Journal of Chemical and Pharmaceutical Research. 4(1): 360-364.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82(2): 291–295.

Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P. and Gargova S. (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). Food Chemistry. 102: 764-770.

Sofowora, A. (1993). Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa.Spectrum Books Ltd. Ibadan Nigeria.

Suhaj, M. (2006). Spice antioxidants isolation and their antiradical activity: a review. Journal of Food Composition and Analysis. 19: 531-537.

Rafatullah, S. M., Tariq, M. A., Al-yahra, J. S., Mossa and Ageel A.M. (1990). Evaluation of turmeric (*Curcuma longa*) for gastric and duodenal antiulcer activity in rats. J. Ethnopharmacol. 29: 25-34.

Rajpal V. (2005). Standaraization of Botanical. Eastern Publishers. 2: 361.

Roopashree, T. S., Dang, R., Rani, R. H. S. and Narendra, C. (2008) Antibacterial activity of antipsoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. International Journal Applied Research in Natural Products. 1(3): 20–28

Valko, M., Leibfritz, D., Moncola, J., Cronin, M.T.D., Mazura, M. and Telser J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. International Journal Biochemistry Cell Biology. 39: 44–84.

Zheng, W. and Wang, S. (2001). Antioxidant activity and phenolic composition in selected herbs. Journal of Agricultural and Food Chemistry. 49: 5165-5170.