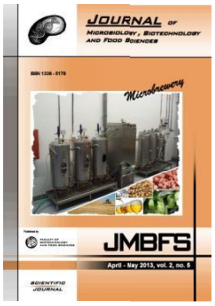

JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

Kumar Roy et al. 2013 : 2 (5) 2283-2290

QUALITY COMPOSITION AND BIOLOGICAL SIGNIFICANCE OF THE BANGLADESHI AND CHINA GINGER (*ZINGIBER OFFICINALE* ROSC.)

Sudam Nandi[1], Md. Moshfekus Saleh-e-In[2], Md. Matiur Rahim[2], Md. Nurul Huda Bhuiyan[2], Nasim Sultana[2], Md. Aminul Ahsan[2], Shamim Ahmed[2], Shajahan Siraj [2], Md. Zamilur Rahman[2], Sudhangshu Kumar Roy*[2]

Address(es): Sudhangshu Kumar Roy
[1]Department of Chemistry, Jagannath University, Dhaka-1100, Bangladesh.
[2]Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh.

*Corresponding author: saleheen_sosthe@yahoo.com

ARTICLE INFO

Received 27.9.2012
Revised 9.2.2013
Accepted 13.2.2013
Published 1.4.2013

Regular article

OPEN ACCESS

ABSTRACT

The essential oil of *Zingiber officinale* Rosc. was extracted from China and Bangladeshi varieties and yielded 0.21% and 0.23 % by hydro-distillation method on fresh weight basis respectively. Fifteen compounds were identified and quantified by GC-MS. The major constituents of China and Bangladeshi ginger essential oils were zingiberene 38.10 % and 41.49%, β-phellandrene 12.0% and 9.92%, α-citral 11.48% and 9.76 %, α-curcumene 9.22% and 11.58%, camphene 5.94% and 4.60% , β-bisabolene 4.39% and 5.0% respectively. The IC_{50} (DPPH method) values were found 61.18 µg/mL and 56.71 µg/mL with the highest inhibition of 78.49 % and 80.77% and the LC_{50} values in the brine shrimp lethality cytotoxicity bioassay were found 0.4842 µg/mL and 0.7151 µg/mL in China and Bangladeshi ginger essential oil respectively. Both the essential oils showed significant activities against some gram positive, gram negative bacteria and fungi. The proximate composition of the China and Bangladeshi variety showed the ash (7.12±0.151, 8.15±0.18%), protein (5.47±0.19, 6.60±0.16%), crude fibre (4.32±0.10, 4.61±0.12%), carbohydrate (16..06±0.35, 18.38±0.41) and food energy (70.50±0.89, 81.74±1.01 kcal/100g.) respectively. The elemental compositions of the both varieties were found rich in Ca, Mg, Fe, Al, Se, Na and K. These results indicate the quality composition of the two varieties may find interest in spice and culinary industries as well as in medicinal preparation.

Keywords: *Zingiber officinale*, essential oil, limonene, GC/MS, mineral element, antimicrobial, antioxidant, cytotoxicity

INTRODUCTION

Spice and medicinal plants gained a more important role in agronomy production, pharmacy and exportation because of their increased use as a raw material for the pharmaceutical industry and in the everyday life (**Abou *et al.*, 2000**). In recent years, research and cultivation of spice plants have been achieved with increasing interest in our country because of the production of good quality spice and also the possibility of exportation.

Zingiber officinale Rosc. commonly known as ginger **(Bengali name Ada),** belonging to the family *Zingiberaceae* of the genus *Zingiber* is comprised of some forty genera and hundreds of species. It is an important culinary aromatic spice for its long standing utility as a flavoring agent. The plant is indigenous to warm tropical climates, particularly South-Eastern Asia. It is also extensively cultivated in India, China, Japan, Australia, Africa, Mexico, Hawaii and Jamaica **(Evans 1989).** The plant is an erect perennial, growing from 1-3 ft. in height. The rhizomes are aromatic thick lobed branched and scaly structures with a spicy lemon-like scent. The essential oil and oleoresins extracted from ginger rhizomes are responsible for the characteristic ginger flavor and pungency. Both oil and oleoresins are used in many food items, soft drinks, beverages, pickles, and many types of medicinal preparations **(Gurdip *et al.*, 2008).** The steam-volatile oil content of dried gingers of commerce ranging from about 1 to 2.5% **(Anonymous 2008).** The essential oil, which is mixture of terpenoid compounds is responsible for the characteristic odorants components. The most predominant important reported components are α-zingiberene, geranial, geraniol, β-bisabolene, nerol, 1,8-cineol, α-terpineol, borneol, p-cineole, α-terpineol β-phellandrene, α-curcumene, α-farnesene, β-sesquiphellandrene, camphene, neral, geranyl acetate, α-terpinene; cadina- 1,4-diene, 6-gingerol and 6-shogaol **(Ghorab *et al.*, 2010; El-Baroty *et al.*, 2010; Moshafi *et al.*, 2009; Zancan *et al.*, 2002; Erler *et al.*, 1988; Ekundayo *et al.*, 1988; Miyazawa *et al.*, 1988; Malek *et al.*, 2005; Sultan *et al.*, 2005; Pino *et al.*, 2004; Kochhar *et al.*, 2003).** Ginger is one of the widely used spices having a long history in traditional oriental medicines as antimicrobial and antioxidant **(Habsah *et al.*, 2000),** anti-inflammatory **(Tjendraputra *et al.*, 2001)**, anti-fungal **(Ficker *et al.*, 2003),** inhibitor of nitric oxide synthesis **(Ippoushi *et al.*, 2003),** antidiarrhoeal activity **(Poonam *et al.*, 2010),** anticonvulsant activity **(Sayyah *et al.*, 2005),** treatment of epilepsy **(Kermani *et al.*, 1988)** and an agent for protecting neuronal cells from amyloidal insult **(Kim and Kim, 2004).** The anticancer properties are reported the presence of pungent vallinoids e.g. 6-gingerol and 6-paradol, shogaols Zingerone, etc. **(Shukla *et al.*, 2006).**

The chemistry of ginger has the subject of sporadic study since the early 19th century. In recent years, the considerable advances on clear understanding of the chemical composition to its quality properties have emerged. There are numerous studies on the composition and activities of ginger essential oil but the comparative study of different growing varieties and quality composition parameters are still lacking. The present paper deals with the chemistry, comparative study of antioxidant, antimicrobial properties and toxic label of ginger essential oil as well as elemental composition of the two varieties.

MATERIAL AND METHODS

Plant material

Two varieties of ginger rhizomes were collected from local supplier. One was imported from China and another one was from Bangladeshi cultivated ginger. The samples were cleaned, freed from dirt and others specimen, air-dried in room temperature, cut into small pieces and stored in airtight high-density double lined polyethylene bag for future research work.

Extraction of essential oils

Ginger was subjected to hydro-distillation using Clevenger's apparatus **(Clevenger 1928)** for 4 h and then dried over anhydrous sodium sulfate and stored at -20°C prior to analysis.

Physico-chemical and proximate studies

The physico-chemical and proximate studies of essential oils and Ginger powder of the two varieties were carried out with three replications by the standard methods **(AOAC 2005; BP 2004; Guenther 1953).**

GC-MS analysis

The essential oils of *Zingiber officinale* Rosc. rhizomes were analyzed by Electron Impact Ionization (EI) method on GC-2010 Shimadzu Gas Chromatograph, coupled to a GC-MS QP 2010 plus Shimadzu Mass Spectrometer; RTX–5 MS fused silica capillary column (Supelco Inc.) (30m x 2.5mm; 0.25 µm film thickness). Column temperature was 40^{o}C (hold 2 min) to 220^{o}C (hold 5 min) at the rate of 10^{o}C/min, maintained with carrier gas helium at a constant pressure of 90 kPa (Acquisition parameters full scan; scan range 40-550 amu). Samples were injected by splitting with the split ratio 10. Mass spectra were taken at 70eV.

Identification of the compounds

The constituents of the oil were identified by calculation of their retention indices under temperature-programmed conditions based on co-injection of homologous n-alkanes (C_6-C_{24}) on RTX-5 MS capillary column. Compounds were identified by comparison of their mass spectra with those of the internal reference mass spectral NIST-107 library.

Antioxidant Activity

The antioxidant activity of the essential oil on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the Brand-Williams method **(Brand-Williams 1995)** with some modifications. In the experiment, 2 mg of each oil was dissolved in methanol and applied by serial dilution technique which concentrations range from 1000 to 1.9531 µg/mL. Two milliliters of a methanol solution of the oil of each concentration was mixed with 3 mL DPPH-methanol solution (40 mg/L) and allowed to stand for 30 min. Then the absorbance was measured at 517 nm using an Analytic Jene Spekel 1300 UV spectrophotometer and from these values, the corresponding percentage of inhibitions were calculated by using the following equation:

$$\% \text{ inhibition} = [\ 1- (ABS_{sample} / ABS_{control})] \times 100$$

Then per cent inhibitions were plotted against respective concentrations. The IC_{50} values were calculated as the concentration of each sample required to give 50% DPPH radical scavenging activity from the graph (linear regression curve). Ascorbic acid and BHT (tert-butyl-1-hydroxytoluene) a potential antioxidant were used as positive control at the concentration of 500 µg/mL-1.953 µg/mL. The experiment was performed triplicate and the results were expressed as mean ±SD with 95% confidence interval in every case.

Antimicrobial Screening

The disc diffusion method **(Bauer 1966; Radovanović 2009).** was used to test antimicrobial and antifungal strains against five gram positive, eight gram negative organisms and three fungi. The bacterial and fungal strains used for the experiment were collected as pure culture from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka. The test samples (8 mg) were made by dissolving in calculated volumes of solvents separately and applied to sterile discs (6 mm diameter) at a concentration of 400 µg/disc and carefully dried to evaporate the residual solvents. Discs containing the test material, were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic ciprofloxacin (5 µg/disc) discs and blank discs (impregnated with solvents) were used as positive and negative control respectively. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter.

Cytotoxicity Activity of Brine Shrimp Lethality Bioassay

In vitro lethality bioassay of the essential oils of ginger rhizome was exploited to detect cytotoxicity by the Mayer's method **(Meyer *et al.*, 1982).** The eggs of Brine Shrimp was collected from local pet shops and hatched in a tank (3.8% w/v sea salt in distilled water) at 30^{o}C in front of a lamp. Eggs were hatched within 48 hours providing large number of larvae (nauplii). The test samples (essential oil) were prepared by dissolving in DMSO (not more than 50 µL in 5 ml solution) and it was applied in 5 mL brine solution (3.8% NaCl in water) to attain concentrations of 0.078 to 40.0 µg/mL. A vial containing 50µL DMSO diluted to 5mL of brine solution was used as a control. Standard vincristine sulfate (VcS) was used as positive control at the concentration of 0.078 µg/mL to 10.0 µg/mL. Then matured shrimps (10 of each vial) were applied to all experimental and control vials. After 24 hours, the vials were inspected using a magnifying glass in front of lamp and the number of surviving nauplii in each vial were counted. The lethal concentrations of essential oil resulting in 50% mortality of the brine shrimp (LC_{50}) from the 24 h counts and the dose-response data were calculated.

Elemental Analyses

Reagents

All reagents used were of analytical-reagent grade. Nitric acid (HNO_3) and perchloric acid ($HClO_4$) were used of trace metal analysis grade (E-Merck Germany). A clean laboratory and laminar-flow hood capable of producing class 100 were used for preparing solution. High purity de-ionized water having a resistivity of 17.5–18.0 MΩ/cm obtained using a Milli–Q water purification system (Millipore, Bedford, MA USA) was throughout. All solutions were stored in high-density polyethylene bottles. Plastic materials were cleaned by soaking in 10% (v/v) HNO_3 for 24h, rinsing three times with de-ionized water and dried in a class 100 laminar flow hood before use. Multi-element stock solutions containing 10 mg/L of each element were obtained from USA (Accu Trace™ Reference Standard, 125 market Street New Haven, CT06513, USA). For the determination of dissolved elements, the samples were filter through 0.45 micron filter paper, then preserved with 1.0% concentrated HNO_3 by volume (0.5 % concentrated HCl is only added for Ag). For the determination of total recoverable elements in unfiltered samples it was acidified with HNO_3 as described above. In all cases, the pH of the samples maintained and verified to be less than 2.0 prior to analysis.

***Ashing procedure and Sample preparation for ICP-MS*:** A certain amount of moisture less sample powder was taken. Ashing procedure and subsequent samples preparation were performed as per AOAC method **(AOAC 2005).**

ICP-MS tuning solution: Contains 10 ppb Ba, Be, Ce, Co, In, Pb, Mg, Tl and Th for instrument tuning and verification of performance.

Metals stock standard: 10 mg/L (Reference / Traceable) of metals Al, As, Ba, Be, Bi, Cd, Ca, Cs, Cr, Co, Cu, Ga, In, Fe, Pb, Li, Mg, Mn, Ni, K, Rb, Se, Ag, Na, Sr, Tl, U, V, Zn.

***Preparation of intermediate (100 µg/L) standard*:** Take 1.0 mL metal stock standard in a 100 mL volumetric flask and dilute up to the mark with 1% HNO_3 diluent.

Preparation of working standard for calibration: Prepare 1, 5, 10, 20 and 50 µg/L working standard from 100 µg/L intermediate standard for carrying out analysis. Take 0.5 mL for 1, 2.5 mL for 5, 5 mL for 10, 10 mL for 20 and 25 mL for 50 µg/L standard solutions from 100 µg/L intermediate standard solution in separate 50 mL volumetric flasks, dilute up to the mark with 1% HNO_3 diluent.

Instrumentation: The ICP-MS measurements were done using a Varian UltraMass™ ICP-MS system (Varian Optical Spectroscopy Instruments, Melbourne Australia). The plasma source was 99.998% argon (Carbagas 3097, Liebefeld, Bern, Switzerland). All operating parameters were under computer control, which allowed simple and fast optimization routines for different matrices. The instrument operating conditions were depicted in Table -1.

Table 1 Operating conditions of Varian ICP-MS

Parameters	Settings
Plasma conditions	
RF power	1.40 kW
Plasma Ar flow rate.	18.0 L min^{-1}
Auxiliary Ar flow rate	2.25 L min^{-1}
Sheath Gas Flow	0.20 L min^{-1}
Nebulizer gas flow	1.0 L min^{-1}
Sampling depth	6.50 mm
Pump Rate	5 rpm
Instrument	
Sampler cone: Nikel	1.0 mm orifice diameter
Skimmer cone: Nikel	0.5 mm orifice diameter

Atomic Absorption Spectrometry Method

The elements Mg, Hg, Fe, Ca, Al were analyzed by Varian Spectra AA 240 FS (Varian Inc. USA) Atomic Absorption Spectrometry. The total mercury in the powder samples (About 2.50 g) were analyzed by a Cold Vapor process (CV-AAS) at a wavelength of 254 nm. coupled with a Varian Vapor Generation (VGA) following the Varian Operation Manual. The quantification limit for the elements were 0.01 µg/kg(ppb). Powder samples were preparation as per AOAC method **(AOAC 2005).**

Flame Photometric Analyses

The concentration of sodium (Na^+) and potassium (K^+) were analyzed by Flame photometer (Jenway PFP-7, England, UK), A graded series of Standard (

ranging from 1-5 ppm) of Na^+ and K^+ solution were used in the serial dilution method for standard curve within linear calibration range and total quantities in samples were calculated.

Data Analysis

The results of antioxidant activity data were transformed into a straight line by means of a trend line fit linear regression analysis by MS Excel version 7 software and cytotoxicity by Biostate 2007 data analysis software. The statistical significants were calculated as p value by SPSS for windows version 13.0 software.

RESULTS AND DISCUSSION

Proximate composition

The proximate compositions of China ginger and Bangladeshi ginger are shown in **Table-2**. China ginger showed higher moisture content (80.42±0.44%) than Bangladeshi ginger (77.30±0.25%). Dry and organic matter contents of both the ginger varieties were calculated on the basis of moisture and ash values respectively. Moisture contents of both the varieties are almost similar to those already reported data **(Peter *et al.*, 1999; Govindrarajan 1982)**. Bangladeshi ginger had higher total ash, water soluble ash, acid insoluble ash, nitrogen, protein, crude fibre, fatty acid, Essential oil, carbohydrate and food energy than the China ginger variety. Protein, crude fibre and carbohydrate contents are the important from the nutritional point of view. As per literature, carbohydrate content reported on fresh weight basis was 7.6±0.679% **(EL-Ghorab *et al.*, 2010)** which might be up to 12.30% depending on varieties **(Anonymous 2008)**, crude fibre content of the fresh ginger reported 8.20±0.36% **(EL-Ghorab *et al.*, 2010)** whereas commercial ginger reported 1.5-6% **(Anonymous 2008).** On the other hand protein contents were reported 7.2±0.09 and 12.40% on fresh weight basis **(EL-Ghorab *et al.*, 2010; Panwar *et al.*, 2005).** The results of protein, crude fibre and carbohydrate contents are significantly higher than the reported values. Because of high moisture contents in our samples, which affect significantly on the constituents. Fatty acid contents were found significantly higher than the earlier reported **(EL-Ghorab *et al.*, 2010; Anonymous 2008)** values (1.4±0.049%, and 0.9%). Essential oil contents of Bangladeshi (0.23%) and China (0.21%) ginger variety determined on fresh weight basis. The results are between the earlier reported data which are 0.31±0.08% and 0.17 % on fresh basis **(EL-Ghorab 2010; Famurewa 2011).** This variation may depend upon the variety, climatic variation and geographical difference. In the case of ash content, dry ginger has been reported 6.64% and 6.1±0.05% **(Peter *et al.*, 1999; EL-Ghorab *et al.*, 2010)** which are comparatively lower values than the current experiment. High amount of water soluble ash contents indicate the presence of highly soluble minerals contents and acid insoluble ash value indicates high digestibility of Bangladeshi ginger variety. The food energy value is emphasizing a little bit high (81.74±1.01) in Bangladeshi variety comparing with the international specification (80.0 kcal) **(USDA 2011).**

Table–2 Proximate composition of Ginger varieties (g/100g)

Parameters	China Ginger	Bangladeshi Ginger
Moisture	80.42±0.44[b]	77.30±0.25[b]
Dry mater	18.10±0.90	22.69±0.25
Organic Matter	92.87±0.156	91.83±0.20
Total Ash	7.12±0.151[a]	8.15±0.18[a]
	1.39±0.09[b]	1.85±0.07[b]
Water soluble Ash	3.74±0.11[a]	4.54±0.17[a]
Acid in soluble Ash	1.24±0.29[a]	1.66±0.19[a]
Nitrogen (N_2)	0.876±0.01[a]	0.97±0.01[a]
Protein	5.47±0.19[a]	6.06±0.16[a]
	1.07±0.01[b]	1.37±0.01[b]
Crude Fiber	4.32±0.10[a]	4.61±0.12[a]
	0.845±0.01[b]	1.04±0.02[b]
Fatty acid	1.13±0.04[a]	2.24±0.05[a]
	0.22±0.01[b]	0.50±0.01[b]
Essential oil	0.21±0.00[b]	0.23±0.005[b]
Carbohydrate	1.52±0.35[a]	1.62±0.23[a]
	16.06±0.35[b]	18.38±0.41[b]
Food Energy (kcal/100g)	37.96±1.29[a]	50.93±0.95[a]
	70.50±0.89[b]	81.74±1.01[b]

Each value represents the average value from three experiments of Mean ±SD, P<0.05 of independent sample T-test. [a] On dry weight basis, [b] on the fresh weight basis.

Physico-chemical properties of the essential oil

The physico-chemical properties of the two varieties essential oils are shown in **Table-3.** Both oils are transparent pale yellow in color with the characteristics odor, bitter taste, freely miscible in some organic solvents and also in 80% ethanol. The essential oil of ginger had specific gravities 0.9033 ± 0.002 and 0.9176 ± 0.002, refractive indexes $[\eta]^{30^\circ C}$ 1.487 ± 0.00 and 1.4877 ± 0.00, optical rotations $[\alpha]_D^{26^\circ C}$ -39.41° ± 0.01 and -38.24° ± 0.04, acid value (mg KOH/g) 7.71±0.31 and 8.53±0.14, ester values (%) 34.37±0.19 and 37.26±0.81 for China and Bangladeshi ginger varieties respectively. The current experimental data are in good agreement with **Govindranath (1982).**

Table 3 Physico-chemical properties of the essential oil of Ginger variety

Characteristics	China Ginger	Bangladeshi Ginger
Appearance (30˚C)	A homogeneous, transparent, pale yellow liquid and lighter then water	
Odor and taste	A spicy odor with bitter taste	
Miscibility and solubility	Insoluble in water but freely miscible in chloroform, carbon tetrachloride, diethyl ether, n-hexane, 100% alcohol, 5.0 volumes in 80% alcohol and 0.1 volumes in 90% alcohol.	
Specific gravity at 30˚C	0.9033 ± 0.002	0.9176 ± 0.002
Refractive index $[\eta]^{30^\circ C}$	1.487 ± 0.00	1.4877 ± 0.00
Optical rotation $[\alpha]_D^{26^\circ C}$	-39.41° ± 0.01	-38.24° ± 0.04
Acid value (mg KOH/g)	7.71±0.31	8.53±0.14
Ester value (%)	34.37±0.19	37.26±0.81

Each value is the mean ± SD of three determinations. P<0.05 of independent sample T-test.

Chemical composition of the essential oil

The chemical compositions of China and Bangladeshi fresh ginger essential oils were identified and quantified by GC-MS and results are presented in **Table-4.** Twelve and ten compounds accounting to 99.99% and 99.91% were present in China and Bangladeshi fresh ginger variety respectively. China ginger essential oil characterized with high amount of monoterpene (33.02%) comprising 20.27% monoterpene hydrocarbon, 12.74% oxygenated monoterpene hydrocarbon and rest of 66.96% sesquiterpene hydrocarbon. The components zingiberene (38.102%), β–phellandrene (12.00%), β-sesquiphellandrene (9.546%), α-curcumene (9.224%), α-citral (7.571%), camphene (5.940%), farnesene (4.573%) and β-bisabolene (4.391%) were found the main components of the China ginger variety. On the other hand Bangladeshi fresh ginger essential oil characterized with high amount of monoterpene (55.19%) comprising with monoterpene hydrocarbon (37.156%), oxygenated monoterpene hydrocarbon (18.034%) and the rest of sesquiterpene hydrocarbon (44.72%). The major components were: zingiberene (26.587 %), camphene (20.605%), β–phellandrene (9.921%), α-curcumene (7.90%), α-citral (9.758%), α-pinene (6.630%), borneol (5.395%), β-sesquiphellandrene (5.242%), and β-bisabolene (4.998%) while farnesene and germacrene-D were absent.

The current results of essential oil compositions of the two varieties are in good agreement with the findings of previous literature, where zingiberene reported the prominent constituents in the fresh ginger oil **(Onyenekwe *et al.*, 1999; Miyazawa *et al.*, 1988; Malek *et al.*, 2005; Singh *et al.*, 2005; Sultan *et al.*, 2005; Moshafi *et al.*, 2009; Antonious *et al.*, 2003).** The chemical compositions of the ginger oil were reported on fresh and dried basis of the different varieties. Australian ginger oil was reported for their high citral contents **(Purseglove *et al.*, 1981)** and the same was reported by **Menut *et al.*, (1994)**. **El-Baroty *et al.*, (2010)** reported β-sesquiphellandrene, **Singh *et al.*, (2008)** identified geranial, **Agrawal *et al.*, (2001)** found curcumene and **El-Ghorab *et al.*, (2010)** reported camphene (15.9%-14.1%) as the prominent constituents in the fresh and dried ginger oil but the variation they found other constituents due to significant effect of drying. The variation of the essential oil composition could be the production and extraction condition, cultivars, variety, stage of mature, adaptive metabolism of plants, agro-climatic condition (climatic, seasonal, geographic) of the seasons and some other factors **(Ghorab *et al.*, 2010; El-Baroty *et al.*, 2010; Singh *et al.*, 2008).** In the current experiment, the variation in the chemical compositions of the two varieties essential oil may be responsible for the said factors.

Table 4 Essential oil composition (% w/w) of Ginger varieties by GC-MS

Compounds	Composition (%)		
	R.I	China Ginger	Bangladeshi Ginger
Camphene[a]	943	5.940	20.605
α- Pinene[a]	948	2.337	6.630
β - Phellandrene[a]	964	12.00	9.921
Cineole[b]	1059	1.265	2.881
Borneol[b]	1138	3.91	5.395
α-Citral[b]	1174	7.571	9.758
β-Sesquiphellandrene[c]	1446	9.546	5.242
Farnesene[c]	1458	4.573	ND
Zingiberene[c]	1451	38.102	26.587
β-bisabolene[c]	1500	4.391	4.998
Germacrene D[c]	1515	1.137	ND
α-Curcumene[c]	1524	9.224	7.90
Total		99.99	99.91

RI: Retention Index, where minimum detection limit is 0.001%, ND stands for not detected. Superscript 'a' for monoterpene hydrocarbon, 'b' for oxygenated monoterpene hydrocarbon and 'c' for sesquiterpene hydrocarbon.

Antioxidant Activity (DPPH radicals)

The antioxidant activity of the different concentration of the fresh ginger essential oil was treated with DPPH radical and comparing with ascorbic acid and BHT. The results of these experiments are shown in Table-5. Ginger oil exhibited marked DPPH free radical scavenging activity in a concentration of 1.953-1000 µg/mL. The IC_{50} values of the two varieties were 61.18±0.75µg/mL and 56.71±0.45µg/mL with the inhibition of 78.49±0.16% and 80.77±0.25% of China and Bangladeshi fresh ginger variety respectively, Hence, the standard BHT and ascorbic acid (as positive control) showed the IC_{50} value of 5.85±0.08 µg/mL and 28.36±0.32 µg/mL respectively. The highest activity was observed in Bangladeshi variety (80.77±0.25) at the 1000 µg/mL dose level. Current results are almost comparable with previous reported data. **El-Baroty *et al.,* (2010**) reported the IC_{50}=65.5µg/mL and **Wei and Shibamoto (2007)** reported the inhibition above 50% at a concentration of 200 µg/mL. Antioxidants react with DPPH to convert it into 1,1-diphenyl-2-picrylhydrazine. DPPH free radical scavenging activity is a concentration dependent manner. The degree of discoloration indicates the radical scavenging potential of the antioxidants **(Singh *et al.,* 2008). Lu and Foo (1995, 2001)** reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidatives properties that create an effective defense system against free radicals. The antioxidant activities observed in ginger oil could be the synergistic effects of two or more compounds present in essential oil **(Singh *et al.,* 2008).** Essential oil of ginger consists of a very complex mixture of various classes of organic compounds, which may produce either synergistic or antagonistic effects on the process of lipid oxidation. Moreover, the antioxidant activity might be responsible of some compounds such as camphene, cineole, borneol and zingiberene **(Wei and Shibamoto 2007).** Therefore, the antioxidative activity might be the such compounds which are present in appreciable amounts of the two varieties.

Table 5 DPPH scavenging activity of Ginger essential oil

Dose Concentration (µg/mL)	Log Concentration (µg/mL)	% of Inhibition (Mean ±SD)			
		China Ginger	Bangladeshi Ginger	Ascorbic Acid(ASA)	BHT
1000	3.0	78.49±0.16	80.77±0.25	--	--
500	2.698	69.77±0.25	69.99±0.33	96.49±0.16	93.38±0.34
250	2.397	65.38±0.53	65.33±0.28	94.05±0.25	88.72±0.25
125	2.096	58.33±0.29	59.61±0.53	93.77±0.34	71.94±0.34
62.5	1.8	50.38±0.25	51.83±0.16	93.05±0.25	59.88±0.25
31.25	1.49	32.66±0.88	34.77±0.58	77.83±0.33	50.83±0.16
15.625	1.19	24.38±0.53	23.83±0.83	70.33±0.50	45.88±0.58
7.8125	0.89	15.88±0.78	21.77±0.25	56.05±0.41	37.33±0.44
3.9062	0.591	11.99±0.16	13.22±0.19	42.44±0.53	30.66±0.16
1.9531	0.290	11.83±0.28	12.27±0.75	39.66±0.57	26.33±0.60
IC_{50} Value (µg/mL)		**61.18±0.75**	**56.71±0.45**	**5.85±0.08**	**28.36±0.32**

ASA = Ascorbic acid, BHT = tert-butyl-1-hydroxytoluene, Mean ±SD, P<0.05 of independent sample T-test.

Antimicrobial Activity

According to the results of disk diffusion assay ginger essential oil are showed **(Table-6)** marked inhibitory effect against all tested strains with the zone of inhibition (15-20 mm) and comparable to ciprofloxacin (CF) as standard antibiotic. In this study *E.coli, S.paratyphi* and *S.dysenteriae* showed the highest activity (20 mm) in Bangladeshi variety than the China variety. On the other hand, *B.mageterium*, *B.subtilis*, *B.sereus* and *S.boydii* showed the minimum activity (15-16mm), Hence, *S.aureus*, *S.lutea*, *P.aureus* and fungi showed the same zone of inhibition (18mm) in the tested samples. Moreover, Bangladeshi ginger oil showed the overall highest inhibitory activity in the tested organism. The results indicate that different antimicrobial species showed different levels of sensitivities on the ginger essential oil and standard. There are several reports on the inhibitory effect of ginger oil on the growth of microorganism **(Janes *et al.,* 1999; Sing *et al.,* 2008). Farag (1989) and Daw (1994)** reported that chemical structure of essential oil or their main compounds such as the presence of functional polar group and aromacity could play an important role for the antimicrobial activity. Therefore, the antimicrobial activity depends on major compound, chemical structure of the compounds and also synergistic action of the all constituents. It was reported that ginger oil rich in sesquiterpenoid compounds and therefore the compounds possessed a wide spectrum antimicrobial activity **(Baratta *et al.,* 1998; Anwar *et al.,* 2009; El-bariti *et al.,* 2010).** In this study, the antimicrobial activity on ginger essential oil could be presence of a major compound zingiberene and also predominant amount of sesquiterpenoid constituents.

Cytotoxicity Activity

Essential oil of *Z. officinale* of the two varieties are shown the most significant cytotoxic activity on brine shrimp larvae **(Table-7)**. China and Bangladeshi ginger essential oil showed the LC_{50} value 0.7951 and 0.8094 µg/mL respectively in comparision to vincrystine sulphate with the LC_{50} value 0.4842µg/mL. No mortality was observed in 50µL/mL DMSO solution. **Sharififar *et al.,* (2009)** and **Moshafi *et al.,* (2009)** reported the significant activity found with the LC_{50} value 10µg/mL and 0.0381µg/mL respectively in the concentration of 10, 100,1000 µg/mL of the ginger essential oil. **Moshafi *et al.,* (2009**) reported the cytotoxicity of the ginger essential oil is responsible for the major compounds zingiberene and β-sesquiphellandrene. The anti-tumar activity of compounds zingiberene and gingerol have been also reported **(Chrubasik *et al.,* 2005).**

It would be inference that, the cytotoxicity of the essential oil of *Z. officinale* might be attributed to these compounds. As a part of our continuous research work, we have evaluated cytotoxicity of essential oil in the safety of herbal spices which have been used widely as condiment. At the high concentration level every flavoring agent or spice must be toxic. The results indicated that, the oil should be used as spice, flavouring in food or condiment in the certain concentration level.

Table 6 Antimicrobial activity of the ginger essential oil (400 µg/disc) and ciprofloxacin (CF) (5 µg/disc)

Test microorganisms		China (mm)	Bangladesh (mm)	CF (mm)
Gram positive bacteria	*Bacillus megaterium*	15	16	45
	Bacillus subtilis	15	16	46
	Staphylococcus aureus	18	18	46
	Sarcina lutea	18	18	45
	Bacillus sereus	16	15	45
Gram negative bacteria	*Escherichia coli*	18	20	46
	Pseudomonas aureus	18	18	46
	Salmonella paratyphi	18	20	46
	Salmonella typhi	17	18	45
	Shigella dysenteriae	18	20	46
	Shigella boydii	15	16	45
	Vibrio mimicus	16	18	46
	Vibrio parahemolyticus	16	18	46
Fungi	*Candida albicans*	18	18	45
	Aspergillus niger	18	18	46
	Sacharomyces cerevaceae	18	18	45

Table 7 Effect of vincriatine sulphate and essential oil on shrimp naupli of *Ginger*

Log Dose (µg/mL)	% of mortality			Probit			LC_{50} (µg/mL)		
	VcS	China	Bangladesh	VcS	China	Bangladesh	VcS	China	Bangladesh
-1.1079	10	10	10	3.72	3.758	3.743			
-0.8069	20	20	20	4.16	4.165	4.161			
-0.5051	50	40	40	5.00	4.750	4.752			
-0.2041	60	60	50	5.25	5.252	5.000			
0.0969	70	60	60	5.52	5.253	5.253	**0.4842**	**0.7951**	**0.8094**
0.3979	80	70	70	5.82	5.524	5.524			
0.699	90	70	80	6.25	5.498	5.841			
1	100	80	80	7.33	5.809	5.790			
1.301	-	90	90	-	6.274	6.258			
1.6021	-	100	100	-	7.158	7.229			

Elemental composition

The elemental compositions of *Z. officinales* of the two varieties are organized in essential and non essential elements and shown in T**able 8(a) and** T**able 8(b)** respectively. The concentrations of the elements are calculated on fresh (FB) and dry weight basis (DB) within the significance level ($p \leq 0.05$).

Essential elements

The mineral contents of Na (100.04 mg/kg), K (6.32 g/kg), Mg (0.56 g/kg) and Ca (0.472 g/kg) were found the highest in the Bangladeshi ginger variety than the China variety, while Ca (0.509 g/kg) was the highest in China variety on fresh weight basis. In comparison to published elemental data, Na (66.0 mg/kg DB) **(Ismail *et al.*, 2011),** K (21723±19 mg/kg DB, 2248 mg/kg DB and 4150 mg/kg FB) **(Olabanji *et al.*, 2007; Sheng-bang *et al.*, 2005; USDA 2011),** Mg (600 mg/kg DB, 22.4 mg/kg FB and 1613 mg/kg DB **(Famurewa *et al.*, 2011; Ismail *et al.*, 2011; Sheng-bang *et al.*, 2005),** Ca (1100 mg/kg DB and 160 mg/kg DB) **(Famurewa 2011; USDA 2011)** and Fe (32.0 mg/kg DB , 6 mg/kg FB, 34.0±1.1 mg/kg DB and 135.2 mg/kg DB) **(Ismail *et al.*, 2011; USDA 2011; Ozkutlu *et al.*, 2006; Sheng-bang *et al.*, 2005)** were lower than the current findings. On the other hand, the reported values of Na (130 mg/kg FB) **(USDA 2011),** Mg (3217±48 mg/kg FB), Ca (3337±42 mg/kg FB) and Fe (663±4 mg/kg DB) **(Olabanji *et al.*, 2007)** were higher than the current results. These mineral elements play a significant role in human metabolism and life process.

The elements of Be (1.27), Ga (12.04), Al (79.23), Se (408.79), Ag (3.22) and Cs (3.94), were observed the highest amounts in the Bangladeshi ginger variety, whereas Li (68.74), Co (7.79) and Bi (3.93) were found the highest in the China variety in µg/kg on fresh weight basis. Hence, Ni was not found in the China variety. A comparisons with reported data, Co (1.073, 0.480 mg/kg DB) **(Ismail *et al.*, 2011; Lamari *et al.*, 2011),** Ni (0.06 , 6.7±1.07, 2.37±0.61 mg/kg DB) **(Ismail *et al.*, 2011; Olabanji *et al.*, 2007; Ziarati *et al.*, 2012)** and Be (32.7 ±11.7 mg/kg DB) (**Olabanji *et al.*, 2007)** contents were higher than the current experimental results. In the case of Se contents (188.97 and 408.79 µg/kg FB) which are interestingly higher than the **USDA (2011)** specification (7.0 µg/kg FB). Se has got anti-oxidising function and it is essential for providing the organism with triiodothyroxine produced from thyroxine **(Forrer *et al.*, 1998).** It contributes to the maintenance of cellular antioxidative balance when taken up at the recommended dietary allowance of 50-100 µg/day and tolerable upper intake limit is 400 µg/day **(FNB 1989).** The high dosage of selenate has been shown to normalize hyperglycemia **(Chowdhury *et al.*, 2007).** The current values of Al were found higher than the reported (0.22 mg/kg DB **(Alam *et al.*, 2008)** value. The importance of Co is the component vitamin B12. The Daily Diatary Intake (DDI) of Co is 7-50 µg/day and the maximum level is 0.3 mg **(Lesnicewcz *et al.*, 2006).** Ni plays an important role in the production of insulin and mostly present in the pancreas. The recommended daily intake should be less than 1 mg/day beyond which it is toxic **(Ziarati *et al.*, 2012).** The elements of Li, Be, Ga, Ag, Cs and Bi were found the first time in Bangladeshi sample by ICP-MS. These elements play important role in the physiological functioning in the body. These elements are essential constituents of enzymes and play a vital role in human metabolism. The human body cannot synthesize them and they must be supplied by food. Essential elements in spices may be correlated with their taste and adding spice in food not only increase flavor but also enhance its nutritive value by providing several essential nutrients in bioavailable form **(Ila and Jagun 1980; Sing *et al.* 2006).** However excessive intake limit is the cause of the toxic effect in the body.

Non-essential elements

In recent years, much emphasis is being laid on Non-essential or toxic elemental contents, in several countries in respect of spice and medicinal plants. **Table-8(b)** showed the toxic elemental contents of the Bangladeshi and China variety. As (45.61) and Pb (101.99) contents were present higher amount in the Bangladeshi variety than the China variety (µg/kg FB). On the other hand Cd (7.75) and Hg (113.86) contents were found higher in China variety than Bangladeshi variety in µg/kg on fresh basis. Cd 0.072±4.1, 0.12±0.01, 1.64±0.11 mg/kg DB **(Ozkutlu et al., 2006; Alam *et al.*, 2008; Ziarati *et al.*, 2012),** Pb 7.06±0.21 and 6.0 mg/kg DB **(Ziarati *et al.*, 2012; Sheng-bang *et al.*, 2005)** and Hg 0.58±0.06 mg/kg DB **(Ziarati *et al.*, 2012)** were also found in the earlier reported data. Pb is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration **(ATSDR 2007).** Cd intoxication can lead to kidney, bone and pulmonary damages **(Godt *et al.*, 2006)**. Hg poisoning can result in damage to the brain, kidney, and lungs. including acrodynia (pink disease), Hunter-Russell syndrome and Minamata disease **(Jack *et al.*, 2007).** The acute sign of As poisoning includes fever, anorexia, hepatomegaly, cardiac arrhythmia, transient encephalopathy and irritation of the gastrointestine tract **(Anonymous 1998).** The WHO maximum limit for Pb and Cd prescribed in herbal medicines and products is 10 mg/kg and 0.3 mg/kg while the dietary intake limits are 3 and

10.3 mg/kg respectively **(WHO 1998).** On the other hand the permissible limits of Hg and As are 1 and 10 mg/kg in foodstuff respectively **(FDA 1999).** The results of toxic elemental contents were found below of the permissible limit throughout the study.

Element uptaken by a plant is its characteristic property and may depend on the use of fertilizers, irrigation water and different climatologically or geo-environmental factors. **(Chowdhury *et al.*, 2007).** In the current study, the elemental composition of ginger vary from one variety to other due to the said factors.

Table 8(a) Essential elemental composition of Ginger variety

Elements (µg/kg)	China		RSD %	Bangladesh		RSD %
	DWB	FWB		DWB	FWB	
Li	379.78	68.74	2.36	207.35	47.05	2.01
Be	3.67	0.66	18.82	5.60	1.27	17.31
Co	43.04	7.79	1.28	23.69	5.38	0.35
Ni	ND	0	ND	558.58	126.74	0.28
Ga	51.13	9.25	0.48	53.09	12.04	1.69
Ag	6.47	1.17	0.79	14.16	3.22	0.73
Cs	12.48	2.26	5.71	17.35	3.94	1.57
Bi	21.69	3.93	95.27	9.91	2.25	2.36
Se	1044.06	188.97	11.54	1801.62	408.79	6.12
Na (mg/kg)	474.13±0.6	85.82±0.10	-	440.19±0.8	100.04±0.18	-
Fe (mg/kg)	215.04	38.92	0.1	195.55	44.37	0.1
Al (mg/kg)	269.18	48.72	0.1	349.20	79.23	0.1
K (g/kg)	16.97±8.6	3.07±1.6	-	27.86±12.6	6.32±2.85	-
Ca (g/kg)	2.81	0.509	0.1	2.08	0.472	0.1
Mg (g/kg)	2.762	0.49	0.1	2.476	0.56	0.1

ND: Not Detected, RSD: Relative Standard Deviation, $p \leq 0.05$.

Table 8(b) Non-essential elemental composition µg/kg of Ginger variety

Elements	China		RSD %	Bangladesh		RSD %
	DWB	FWB		DWB	FWB	
As	55.66	10.07	89.47	201.03	45.61	1.09
Hg	629.07	113.86	0.1	355.52	80.67	0.1
Pb	331.61	60.02	32.77	449.49	101.99	1.69
Cd	42.80	7.75	2.77	14.67	3.33	1.36

RSD: Relative Standard Deviation, $p \leq 0.05$.

CONCLUSION

Ginger is usually mixed with tea, beverage and also used as spice for flavoring purposes all over the world. The essential oil composition of ginger variety showed rich in sesquiterpene and monoterpene hydrocarbon compounds. The biological studies of the oil possessed the high antioxidative activity as well as antimicrobial effect. This may be used in food industries as preserver of food product against spoilage by bacteria and fungi. Nineteen elements were detected at various concentration levels. These elements are the meaningful sources of major and trace elements in the human nutrition. The proximate and mineral compositions of Bangladeshi and China ginger in respect of their nutritional values are in good agreement with USDA quality standard. On the other hand, toxic elemental compositions were found below the recommended toxic level, whose consumption is not harmful as per recommended doses. The results of these studies imply that Bangladeshi ginger variety complies with the

international quality standard and it may be used as spice and in food industries as well as in medicinal preparation.

Acknowledgments: Authors are thankful to the Director, IFST BCSIR, Dhaka and the Director, BCSIR Laboratories, Dhaka, Bangladesh for giving the permission to conduct the research work. Authors are also thankful to Biomedical Research Centre, University of Dhaka, Bangladesh.

REFERENCES

ABOU ARAB, A.A., ABOU DONIA, M.A. 2000. Heavy metals in Egyptian spices and medicinal plants and effect of processing on their levels. *J. Agric. Food Chem*., 48(6), 2300-2304.

AGRAWAL, M., WALIA, S., DHINGRA, S., KHAMBAY, B.P.S. 2001. Insect growth inhibition, antifeedant and antifungal activity of compounds isolated/derived from Zingiber officinale Roscoe (ginger) rhizomes. *Pest Manag. Sci*., 57, 289-300.

ALAM, S., BANGASH, F.K., AHMED, I. ZIAUDDIN. 2008. Comparative study for the determination of selected nutrients in Solanum tuberosum L. and Zingiber officinale Roscoe. *Journal of Chemical Society Pakistan*, 30(4), 588-593.

ANONYMOUS. 1998. Dictionary of Toxicology. Ernest Hodgson, Janice E. Chambers and Richard B. Mailman Edited Second edition, London : Macmillan Reference ; New York, NY, USA : Published in the US and Canada by Grove's Dictionaries, p.55.

ANONYMOUS. 2008. Chemistry of Spices, Villupanoor A. Parthasarathy, Bhageerathy Chempakam, T. John Zachariah Edited, CAB International , Wallingford 7th Floor, Oxfordshire OX10 8DE Cambridge, MA 02139, UK. pp.70-96.

ANTONIOUS, G.F., KOCHHAR, T.S. 2003. Zingiberene and curcumene in wild tomato. *Journal of Environmental Science and Health Part B, Pesticides, Food Contaminants, and Agricultural Waste*, 38(4), 489–500.

AOAC. 2005. Official Methods of Analysis. Association of Official Analytical Chemist. 14th Ed, Sydney, Williams. Arlington, USA. pp. 503-532.

ATSDR. 2007. Agency for toxic Substances and Disease Registry. Toxicological Profile for Lead. U.S. Department of Health and Human services, Public Health Service, Atlanta, GA, United States

BP. 2004. British Pharmacopoeia, Package Ed. British Pharmacopoeia Commission (Stationery Office Books). IVA pp. 248-250.

BARATTA, M.T., DORMAN, H.J., DEANS, S.G., FIGUEIREDO, A.C., BARROSO, J.G., RUBERTO, G. 1998. Antimicrobial and antioxidant properties of some commercial oil. *Flav. Frag. J.,* 13, 235-244.

BAUER, A.W., KIRBY, W.M.M., SHERRIS, J.C., TURCK, M. 1966. Antibiotic susceptibility testing by standardized single disc method. *Am.J.Clin. Pathol.*, 44, 493-496.

BRAND-WILLIAMS, W., CUVELIER, M.E., BERSET, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.*, 28, 25-30. (doi:10.1016/S0023-6438(95)80008).

CHRUBASIK, S., PITTLER, M.H., ROUFOGALIS, B.D. 2005. Zingiberis : A comprehensive review on the ginger effect and efficacy profile. *Phytomedicine*, 12, 84-701.

CLEVENGER, J.F. 1928. Apparatus for the determination of volatile oil*.,* 17, 346 p.

CHOUDHURY, R.P., GRARG, A.N. 2007. Variation in essential, trace and toxic elemental contents in Murraya koenigii-A spice and medicinal herb from different Indian states. *Food Chemistry*, 104(4), 1454-1463.

DAW, Z.Y., EL-BAROTY, G.S., MAHMOUD, A.E. 1994. Inhibition of Aspergillus parasiticus growth and alfatoxin production by some essential oils. *Chem. Mikrobial. Technol. Lebensm.,* 16(5/6), 129-135.

EI-GHORAB, A.H., NAUMAN, M., ANJUM, F.M., HUSSAN S., NADEEM, M. 2010. A comparative study on chemical composition and antioxidant activity of Ginger (Zingiber officinale) and cumin (Cumunum cyminum). *J.Agric. Food. Chem.,* 58(14), 8231-8237.

EKUNDAYO, O., LAAKSO, I., HILTUNEN, R. 1988. Composition of ginger (Zingiber officinale Roscoe) volatile oils from Nigeria. *Flavour and Fragrance Journal*, 3(2), 85–90.

EL-BAROTY, G.S., EL-BAKY ABD, H.H., FARAG, R.S., SALEH, M.A. 2010. Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils. *African Journal of Biochemistry Research*, 4(6), 167-174.

ERLER J., VOSTROWSKY O., STROBEL H. AND KNOBLOCH K. 1988. Essential oils of ginger, Zingiber officinalis Roscoe. *Zeitschrift fur Lebensmittel Untersuchung und Forschung*, 186(3), 231–234.

EVANS, W.C. 1989. Trease and Evans. Pharmacognosy. 13th ed. Bailliere Tindall Press, London, pp.216-217.

FAMUREWA, A.V., EMUEKELE, P.O., JAIYEOBA, K.F. 2011. Effect of drying and size reduction on the chemical and volatile oil contents of ginger (Zingiber officinale). *Journal of Medicinal Plants Research*, 5(14), 2941-2944.

FARAG, R.S., DAW, Z.Y., ABO-RAYA, S.H. 1989. Influence of some essential oils on Aspergillus parasiticus growth and alfatoxins production in a synthetic medium. *J. Food Science*, 54, 74-77.

FDA (1999) Food and Drug administration Code of Fed Reg, 40 CFR 101, Washington DC.

FICKER, C., SMITH, M.L., AKPAGANA, K., GBEASSOR, M., ZHANG, J., DURST, T., ASSABGUI, A., ARNASON, J.T. 2003. Bioassay-guided isolation and identification of antifungal compounds from ginger. *Phytotherapy Research*, 17, 897-902.

FORRER, R., GAUTSCHI, K., STROH, A., LUTZ, H. 1998. Direct determination of Selenium and other trace elements in serum samples by ICP-MS. *Journal of Trace Elements in Medicine and Biology*, 12, 240-247.

GODT, J., SCHEIDIG, F., GROSSE-SIESTRUP, C., ESCHE, V., BRANDENBURG, P. 2006. The Toxicity of Cadmium and resulting hazards for human health. *J Occup Med Toxicol*., 1, 22 p.

GOVINDARAJAN, V.S. 1982. Ginger: Chemistry, technology and quality evaluation: Part 1.CRC critical reviews. *Food Sci Nutr*., 17(1), 1-96.

GUENTHER, E. 1953. The Essential Oils, Vol-I, 1st Edn., (D. Van Nostrand Co. Inc. New York pp. 249-293.

GURDIP, S., KAPOOR, I.P.S., PRATIVA, S., HELUANI, C.S.D., MARINA, P.L., CESAR, A.N.C. 2008. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of Zingiber officinale. *Food and Chemical Toxicology*, 46, 3295-3302.

HABSAH, M., AMRAN, M., MACKEEN, M.M., LAJIS, N.H., KIKUZAKI, H., NAKATANI, N., RAHMAN, A.A., GHAFAR ALI, A.M. 2000. Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. *Journal of Ethnopharmcology*, 72, 403-410.

ILA, P., JAGAN, P. 1980. Multielemental analysis of food spices by instrumental neutron activation analysis. *Journal of Radio Analytical Chemistry*, 57, 205-210.

ISMAIL, F., ANJUM, M.R., MAMON, A.N., KAZI, T.G. 2011. Trace metal contents of vegetables and fruits of Hyderabad retail market. *Pakistan Journal of Nutrition*, 10(4), 365-372.

JACK, C., CLIFTON, M.S. 2007. Mercury Exposure and Public Health. *Pediatric Clinics of North America*, 54(2), 237-237.

JANES, M.E., NANNAPANENT, R., JOHNSON, M.C. 1999. Identification and characterization of two bacteriocin producing bacteria isolated from garlic and ginger root. *J Food Prot*., 62, 899-904.

KERMANI, M.K.K. 1988. Daghayegh-ol-Eladj, Saadat Press, Kerman p.365.

KIM, D.S.H.L., KIM, J.Y. 2004. Side-chain length is important for shogaols in protecting neuronal cells from β-amyloid insult. *Bioorganic and Medicinal Chemistry Letter*, 14, 1287-1289.

LAMARI, Z., LARBI, R., YOGOUBI, B. 2011. Trace element content of ginger and sage medicinal plants from Algeria. *Health*, 3(9), 542-544.

LESNIEWICZ, A., JAWORSKA, K., ZYRNICKI, W. 2006. Macro and micro nutrients and their bioavailability in polish herbal medicaments. *Food Chemistry*, 99, 670-679.

LU, F., FOO, L.Y. 1995a. Antioxidant activity from polyphenol from sage (Salvia officinalis). *Food Chem*., 75, 197-202.

LU, F., FOO, L.Y. 1995b. Phenolic antioxidant components of evening primorose. In : Ong, ASH, Niki E, Packer L (Eds), Nutritional Lipids, Health and Diseases. AOAC, Champoin, IL.

MALEK, ABD, S.N., IBRAHIM, H., HONG, S.L., LEE, G.S., CHAN, K.S., MOHD ALI, N.A. 2005. The essential oils of Zingiber officinale variants. *Malaysian Journal of Science*, 24(2), 37–43.

MENUT, C., LANATY, G., BESSIERE, J.M., KOWDAV, J. 1994. Aromatic plants of tropical Central Africa. XIII. Rhizomes volatile components of two *Zingiberales* from the Central Africa Republic. *J. Essent Oil Res*., 6, 101-108.

MEYER, B.N., FERRIGNI, N.R., PUTNAM, J.E., JACOBSEN, J.B., NICHOLSAND, D.E., MCLAUGHLIN, J.L. 1982. Brine shrimp; a convenient general bioassay for active plant constituents. *Planta Medica*, 45, 31-34.

MIYAZAWA, M., KAMEOKA, H. 1988. Volatile flavour components of Zingiberis Rhizoma (Zingiber officinale Roscoe). *Agricultural and Biological Chemistry*, 52(11), 2961–2963.

MOSHAFI, M.H., FARIBA, S., DEHGHAN, R.G., AMERI ALI, E.H. 2009. Bioassay screening of the essential oil and various extracts of fruits of Heracleum persicum Desf. and Rhizomes of Zingiber officinale Rocs. using Brine Shrimp cytotoxicity assay. *Iranian Journal of Pharmaceutical Research*, 8(1), 59-63.

OLABANJI, S.O., ADESINA, S.K., CECCATO, D., BUOSO, M.C., MOSCHINI, G. 2007. PIXE analysis of some medicinal plants usually estracted and drunk as tea, Beverage, or used as spice or Flavour in Nigeria. Proceedings of the International Conference on PIXE and in Analytical Applications. Puebla, Mexico, p. 25-29.

ONYENEKWE, P.C., HASHIMOTO, S. 1999. The composition of the essential oil of dried Nigerian ginger (Zingiber officinale Roscoe). *European food research and technology*, 209(6), 407-410.

OZKUTLU, F., SEKEROGLU, N., KARA, M. 2006. Monitoring of cadmium and micronutrients in spices commonly consumed in Turkey. *Research Journal of Agricultural and Biological Sciences*, 2(5), 223-226.

PANHWAR, F. 2005. Ginger (Zingiber officinale Rosc.) cultivation in Sindh Pakistan; Digital Verlag GMBH Publishing: Germany.

PETER, K.V., KANDIANNAN, K. 1999. Ginger; Tropical Horticulture, Vol.1; Bose T.K, Mitra S.K.; Farooqi A.A. Sadhu M.K. Eds; Naya prokash; Calcuta.

PINO, J.A., MARBOT, R. 2004. Rosado A and Batista A. Chemical composition of the essential oil of Zingiber officinale Roscoe L. from Cuba. *Journal of Essential Oil Research*, 16(3), 186–188.
POONAM, G., DASWANI, S.B., PUNDARIKAKSHUDU, T., NOSHIR, H.A., TANNAZ, J.B. 2010. Antidiarrhoeal activity of Zingiber officinale (Rosc.). *Current Science*, 98(2), 222-229.
PURSEGLOVE, J.W., BROWN, E.G., GREEN, C.L., ROBBINS, S.R.J. 1981. Spice, Volume -2. Longman, New York, pp-447-531.
SAYYAH, M., MOAIED, S., KAMALINEJAD, M. 2005. Anticonvulsant activity of Heracleum persicum seed. *Journal of Ethnopharmacol.*, 98, 209-211.
SHARIFIFAR, F., MOSHAFI, M.H., NUDEHE, G.D., AMERI, A., ALISHAHI, F., POURHEMATI, A. 2009. Bioassay screening of essential oil and various extracts from 4 spices medicinal plants. *Pak. J. Pharm. Sci.,* 22(3), 317-322.
SHENG-BANG, Z., YU-SHENG, G. 2005. ICP-AES determination of trace elements in dried ginger. *Analysis and inspection of Food Science*, 26(9), 395-396.
SHUKLA, Y., SINGH, M . 2006. Cancer preventive properties of ginger: a brief review. *Food and Chemical Toxicology*, 45(5), 683–690.
SING, V., GRAG, A.N. 2006. Availability of essential trace elements in Indian cereals, vegetables and spices using INAA and the contribution of spices to daily dietary intake. *Food Chemistry*, 94, 81-89.
SINGH, G., MAURYA, S., CATALAN, C., LAMPASONA, M.P. 2005. Studies on essential oils, part-42, Chemical antifungal, antimicrobial and sprout suppressant studies on ginger essential oil and its oleoresin. *Flav. Frag J.*, 20, 1-6.
SULTAN, M., BHATTI, H.N., IQBAL, Z. 2005. Chemical analysis of essential oil of ginger (Zingiber officinale). *Pakistan Journal of Biological Sciences*, 8(11), 1576–1578.
TJENDRAPUTRA, E., TRAN, V.H., LIU-BRENNAN, D., ROUFOGALIS, B.D., DUKE, C.C. 2001. Effect of ginger constituents and synthetic analogues on cyclooxygenase-2 enzyme in intact cells. *Bioorganic Chemistry*, 29, 156-163.
USDA (United State Department of Agriculture), Agricultural Research Service. (2011). USDA National Nutrient Database for Standard Reference, Release 24. Nutrient data for 11216 ginger root Raw. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl
WEI, A., SHIBAMOTO, T. 2007. Antioxidant activity and volatile constituents of various essential oils. *J. Agric. Food Chem*., 55, 1737-1742.
WHO. 1998. World Health Organization. Quality Control Methods for Medicinal Plant Materials. WHO Geneva Swizerland.
ZIARATI, P. 2012. Determination of contaminants in some Iranian popular herbal medicines. *Environmental and Analytical Toxicology*, 2, 120 p.