Minerals Evaluation, Antibacterial Activities and Antioxidant Activities of *Zingiber officinale* Roscoe (ချင်း)

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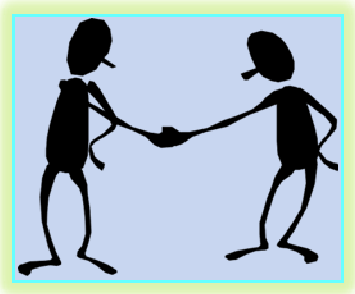
Introduction



medicinal plants extracts- contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potential[(1)]

80% of the population of developing countries depend on traditional medicines, mostly natural plant products

minimally toxic, cost effective and pharmacologically active, and provide an easy remedy

synthetic drugs, which are a subject of adulteration and side effects[(2)]

Minerals- essential to human nutrition are accumulated in different parts of plants[3]

Plants may absorb minerals from soil, water or air [4]

micronutrient malnutrition is a major global health concern

half of the total population in developing countries are reported to be affected by micronutrient deficiency [5]

new antibiotics and plant based antimicrobial compounds are effective against the resistant organisms [7]

alarming increase in the rate of infection by antibiotic resistant microorganisms has urged scientists to search for compounds which have potential antimicrobial activity

plant extracts on microorganism - studied worldwide[2].

Antioxidants - vital substances, protect the body from damage caused by free radical induced oxidative stress[8]

Free radicals - depletion of immune system antioxidants,

change in gene expression and induce abnormal proteins

contribute to many disorders- (atherosclerosis, arthritis, ischemia, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer, and AIDS)[9]

Antioxidant property - due to the presence of bioactive compounds (Vitamins, Flavonoids, Terpenoids, Carotenoids, Cumarins, Curcumins, Lignins, Saponin & plant Steroids)[10]

Antioxidants exist within the body - derived from dietary sources like fruits, vegetables and teas[8]

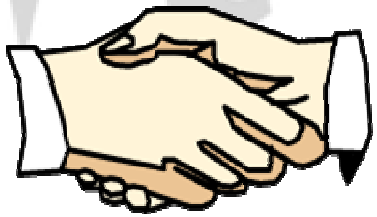
family Zingiberaceae; 45 genera, and 800 species [11]

widely distributed in South-Eastern Asia[12]

consumed dietary condiments in the world

antioxidant, antibacterial, antifungal, anticancer and anti-inflammatory effects [12]

medicinal plants are sources of diverse nutrients and non nutrient molecules, of which many display antioxidant and antimicrobial properties [15]



General objective

To determine minerals evaluation, antibacterial activities and antioxidant activities of *Zingiber officinale* Roscoe, ginger (ချင်း)

Specific objectives

To measure the amount of macrominerals (major elements): calcium, magnesium, potassium and sodium from ginger

To determine the amount of microminerals (trace elements): copper, iron, manganese and zinc from ginger

To find out phytochemical constituents of ginger

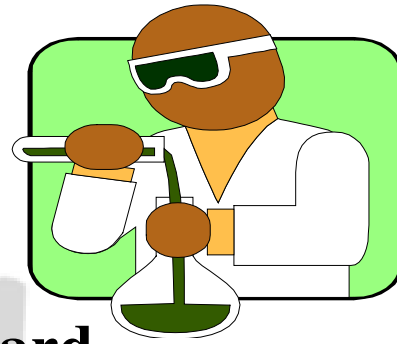
To evaluate the antibacterial activity of ginger extracts on some bacteria

To investigate the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ginger

To determine the antioxidant activities of ginger extracts

Materials and Methods

Reagents and Chemicals



Analytical grade reagents of

Ca, Mg, K, Na, Cu, Fe, Mn and Zn standard

70% Nitric acid (HNO_3)

69% Hydrochloric acid (HCl)

Ethanol

1, 1-diphenyl-2-picryl-hydrazyl (DPPH)

Mueller-Hinton agar

Muller-Hinton broth (Hi Media, India)

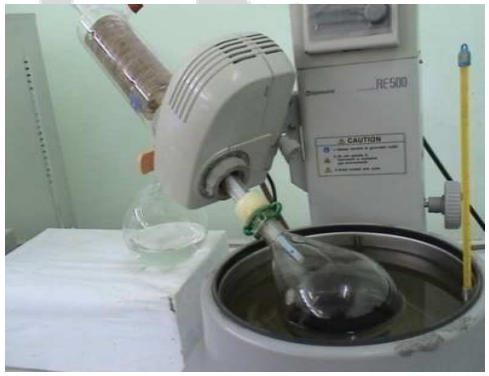
Ceftriaxone 30 µg

Double de - ionized water (DDW)

Instruments

UV visible spectrophotometer UV 1601 PC

Rotary evaporator



Plant authenticity

identified and confirmed for specific botanical name by competent taxonomist

Sample Collection

collected from Mandalay (Mdy) and Pyin Oo Lwin (POL)

Sample preparation

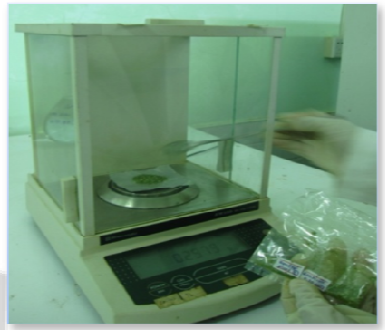
thoroughly washed with tap water and rinsed with distilled water to remove the dust and particles

air dried in shade at room temperature

crushed, powdered and homogenized, using mortar and pestle

dried in oven at 60 ºC to constant weight

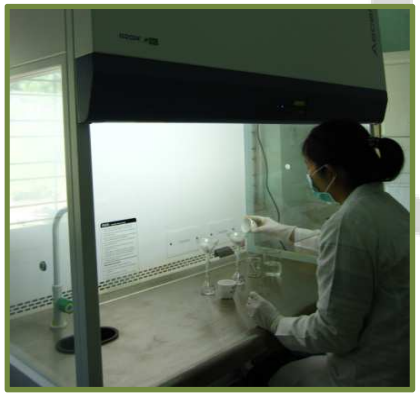
Digestion of Ginger Sample [16]



Ginger powder
(2.5) g

Heating 110 °C, 2 hours, in an oven
to remove moisture

furnace, at 550 °C for 4 hr,
to obtain grey ash, & cool



5 ml of 6M HNO_3 ,
to dissolve & digest, filter

Made up with DDW
(50 ml Volumetric flask)

Blank control was carried out in the same way of sample preparation using solvent alone

Standard solutions of each metal was separately prepared from their respective concentration of 1000 mg/ml stock solutions, from which further serial dilutions were made to cover the optimum absorbance range for standard calibration curve

Reagent blank determination was used to correct the instrument readings

Sample runs was conducted in triplicates[(5)]

Preparation of extracts

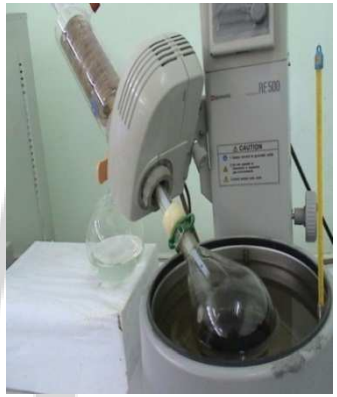
100 g of ginger in solvent

↓ **60°C for 6 hours, in water bath**

extract

↓ **electromantle, 50°C**

dry extract



Phytochemical tests for types of compounds

Harborne J.B (1998) Phytochemical Method [18]

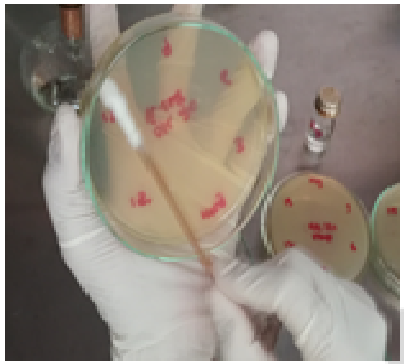


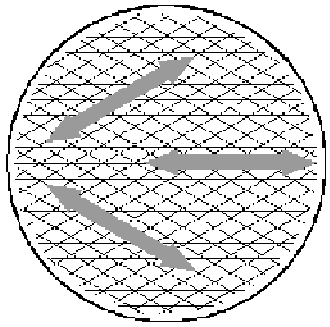

Determination of antibacteriaial activity

- **Determined by agar disc diffusion technique according to modified Kirby and Bauer method (WHO, 2003)**

Bacteria used

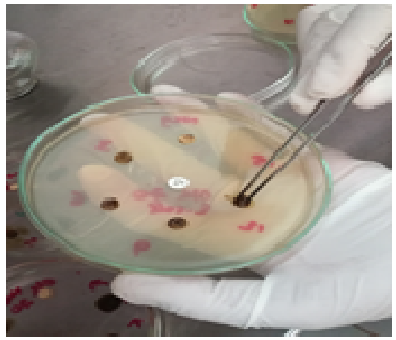
- ***Staphylococcus aureus***
- ***Pseudomonas aeruginosa***
- ***Escherichia coli***

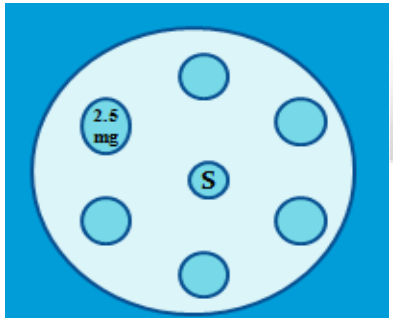




The Mueller Hinton plate were swabbed over the entire surface of the medium 3 times, rotating the plate 60˚after each application

Filter paper discs were applied at equidistance by using sterile forceps





10 µl of each extract was impregnated to discs resulting in the range of 1 mg/disc - 5 mg/disc respectively

Each disc were gently pressed down with sterile forceps to ensure even contact with the medium

The plates were incubated at 37 ˚C for 24 hours

Determination of MIC and MBC

zone of inhibition >7mm were proceeded for minimum inhibitory concentration by broth dilution method

different concentrations of ranging 0.5 -10 mg/ml were tested

series of 11 tubes for each test organisms was prepared

contains 20 µl of test organisms, 1 ml of different concentration of ginger extract and 1 ml of Mueller-Hinton broth

control tube - broth only and inoculum only

incubated at 37^0 C for 24 hours

determination of MBC, one loopful from each tube of above dilutions streaked on Muller Hinton agar plate and incubated at 37° C for 24 hours[20]

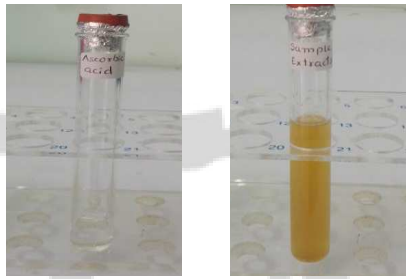
Measurement of DPPH Radical Scavenging Activity by Spectrophotometric method in *in- vitro* models

Preparation of freshly prepared DPPH solution (60 µM)[21]

DPPH (2.36 mg) + 95% ethanol (100 ml)
(60 µM)(Purple)

Final solution in volumetric flask

Preparation of different concentrations of ascorbic acid and ginger extracts



(a) (b)

100 mg/10ml

50% ethanol

100 µg/ml

50% ethanol

Serial dilution

0.5µg/ml 1µg/ml 2µg/ml 4µg/ml 8µg/ml

a = ascorbic acid , b = ginger extract

The scavenging reaction between DPPH and test sample of an antioxidant

30 mins

RT

517 nm

Test sample 2 ml

DPPH 2 ml

Mixing

$$\% \text{ DPPH radical scavenging activity} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

$A_{control}$ = the absorbance of the control solvent (DPPH)

A_{test} = the absorbance in the presence of the tested sample expressed in terms of IC_{50} [22]

Statistical analysis

Microsoft Excel v. 2007

linear regression equation " Y= a X + b"[23]

Results were presented as mean ± SE

Results

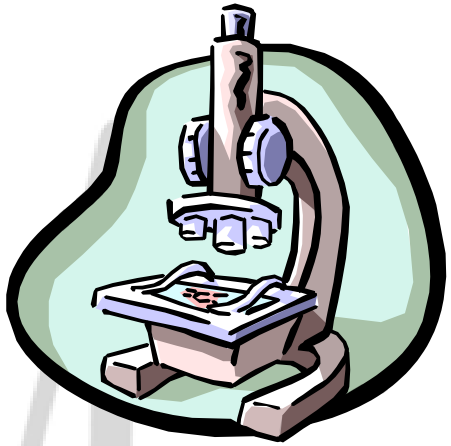


Fig 1: *Zingiber officinale* Roscoe (ချင်း)

family - Zingiberaceae, by an English botanist William Roscoe (1753 – 1831)
perennial, erect, rhizomatus plant up to 3- 4 feet
Rhizome- irregular in shape, brown, pale yellow
Leaves - simple, lanceolate, alternates, distichous
Inflorescence - terminal dense spikes
Flowers - greenish pale yellow

Present/ Other Study	Macrominerals				Microminerals				Reference
	Ca	Mg	K	Na	Cu	Fe	Mn	Zn	
Mdy	605.85 ± 13.85	40.85 ± 0.03	730.58 ± 10.20	85.96 ± 0.79	3.25 ± 0.14	168.65 ± 2.08	145.01 ± 2.14	13.65 ± 0.28	
POL	291.05 ± 8.02	40.61± 0.02	739.14 ± 7.86	83.59 ± 0.90	2.38 ± 0.14	209.57 ± 2.49	168.09 ± 2.09	11.66 ± 0.27	
Pakistan (2011)					49.4 ± 2.7	2457 ± 1110	1014 ± 52	19.7 ± 1.9	25
Ethiopia (2015)	2001-2543	2700-4094			1.10 – 4.78	41.8 – 89.0	184 – 401	38.5 – 55.2	26
India (2008)	1700	9200			4.47	217	313	72.53	27
WHO/ FAO 2001	–	–	–	–	73	425	500	100	28
WHO 1996	3600	–	10-100	400-500	–	–	–	–	34
Ajasa, 2004	44 -614	2000	6380 -36600	2610 -51340	–	–	–	–	30, 31

Extraction of ginger

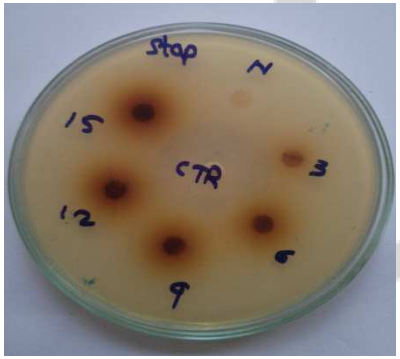
Yield percent

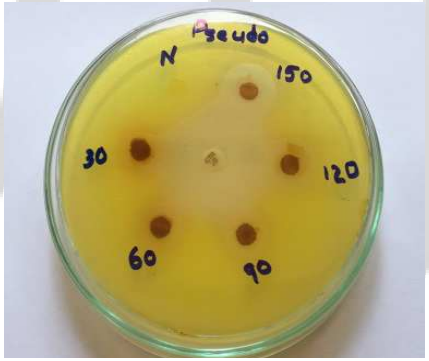
aqueous and 50% ethanolic extracts of
Mdy ginger – 19.51 % , 20.74%
POL ginger – 24.09 % and 32.51%

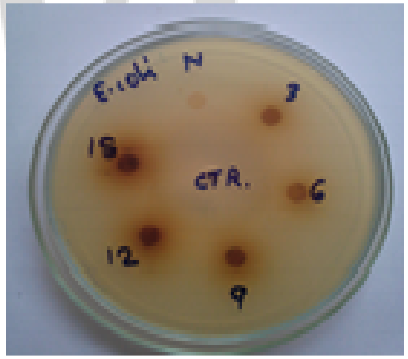
No.	Phytochemical	Reagents	Observed (color)	Ginger	
				Mdy	POL
1.	Alkaloids	Dragendorff's	Orange ppt	+	+
2.	α amino acid	Ninhydrin	Pink color	+	+
3.	Carbohydrate	α – naphthol, Con: H_2SO_4	Pink ring color	+	+
4.	Flavonoids	Con: HCl, Mg	pink reddish, pink brown	+	+
5.	Glycosides	10 % lead acetate	Yellow	+	+
6.	Phenols	10% Fe Cl_3	Blue	-	-
7.	Protein	10 % Na OH, 10 % $CuSO_4$	Red or Violet	-	-
8.	Reducing sugar	Benedict's solution	Brick red	+	+
9.	Saponins	Shaken 15 min	2 cm foam	+	+
10.	Starch	Iodine solution	Blue	-	-
11.	Steroids	Acetic anhydride, Con: H_2SO_4	Green blue	-	-
12.	Tannins	1% Fe Cl_3, Dil: H_2SO_4	Yellowish brown	+	+
13.	Tri-terpene	$CHCl_3$, acetic anhydride, Con: H_2SO_4	reddish brown coloration	-	-

Extracts/ standard	Diameter of inhibition zone (mm) of ginger extracts and Standard		
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Escherichia coli*
Mdy Aq / Ceftriaxone	7 mm /27 mm	13mm /25 mm	12 /28 mm
Mdy EtOH /Ceftriaxone	9 mm /28 mm	15 mm /30 mm	15 /28 mm
POL Aq / Ceftriaxone	8 mm /29 mm	12 mm /17 mm	12 /30 mm
POL EtOH / Ceftriaxone	9 mm /24 mm	15 mm /20 mm	15 /33 mm

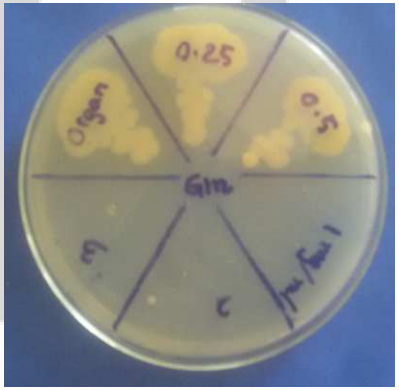
Paper disc – 6 mm, Standard antibiotic – Ceftriaxone 30 µl





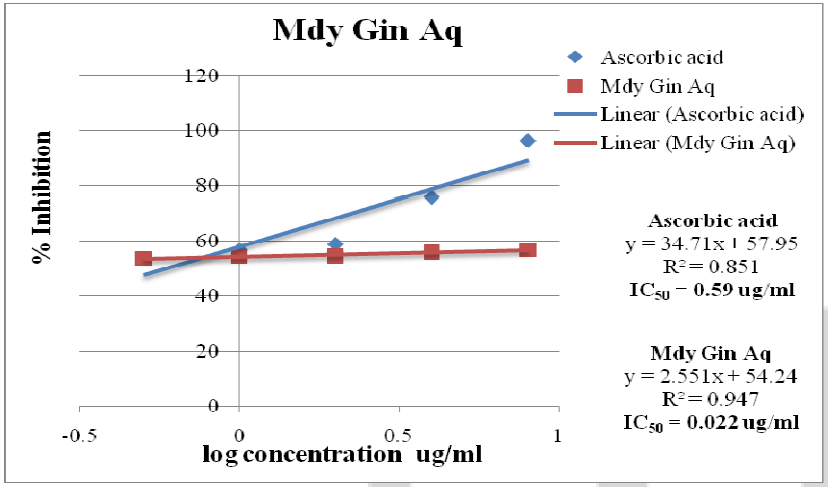


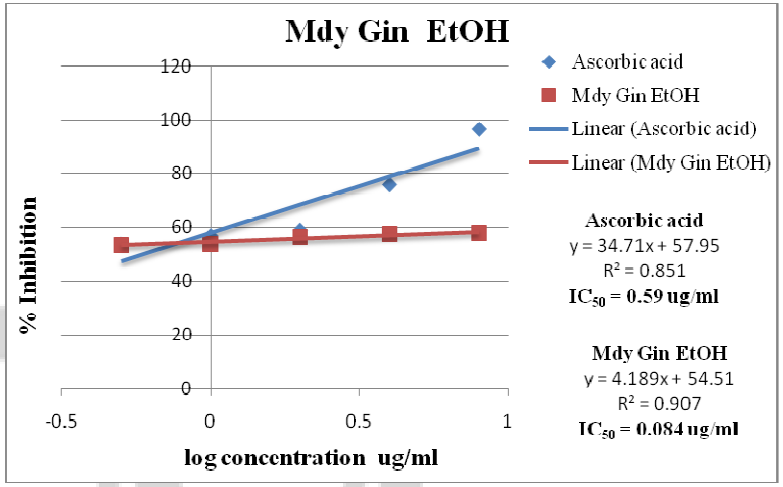
Ginger Extract	*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Escherichia Coli*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	(mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Mdy EtOH	1	2	8	9	3	4
POL EtOH	2	3	8	9	3	4
Mdy Aq	>10	>10	>10	>10	>10	>10
POL Aq	>10	>10	>10	>10	>10	>10

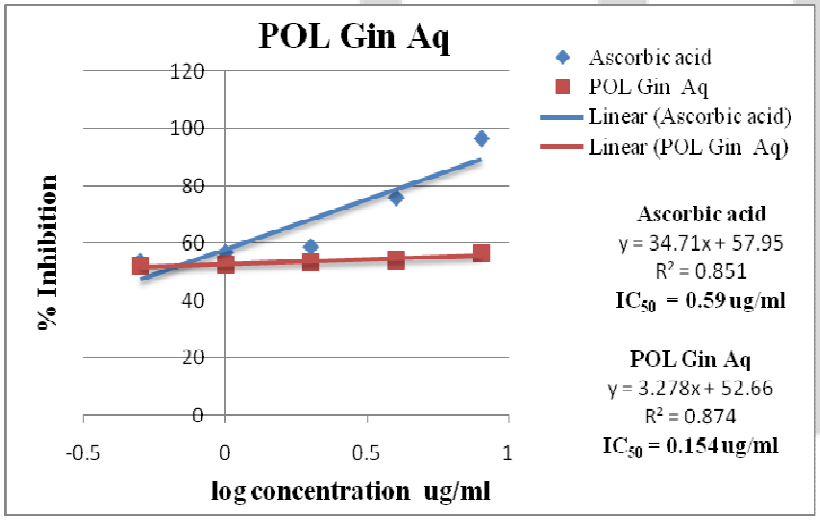


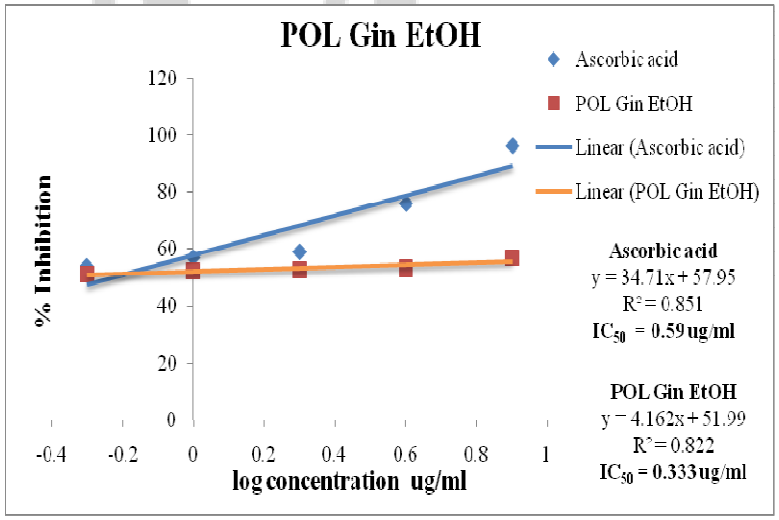
1 = Test organism + Ethanolic extract (0.5 mg/ml)
2 = Test organism + Ethanolic extract (1 mg/ml)
3 = Test organism + Ethanolic extract (2 mg/ml)
4 = Test organism + Ethanolic extract (3 mg/ml)
5 = Test organism + Ethanolic extract (4 mg/ml)
6= Test organism + Ethanolic extract (5mg/ml)
7= Broth Only
8 = Test organism Only

Sr.No	Sample/ Extract		Concentration (µg/ml)					IC_{50} (µg/ml)
			0.5	1	2	4	8	
1	Ascorbic acid	Mean absorbance	0.342	0.319	0.304	0.178	0.026	0.590
		% inhibition	53.78	56.89	58.92	75.95	96.5	
2	Mdy Aqueous	Mean absorbance	0.169	0.167	0.166	0.161	0.158	0.022
		% inhibition	53.69	54.25	54.52	55.89	56.71	
3	Mdy EtOH	Mean absorbance	0.170	0.169	0.159	0.155	0.154	0.084
		% inhibition	53.42	53.70	56.49	57.53	57.81	
4	POL Aqueous	Mean absorbance	0.354	0.352	0.345	0.341	0.323	0.150
		% inhibition	52.43	52.43	53.38	53.92	56.35	
5	POL EtOH	Mean absorbance	0.362	0.353	0.350	0.346	0.319	0.333
		% inhibition	51.1	52.29	52.7	53.24	56.89	


Mdy Gin Aq
% Inhibition
log concentration ug/ml
Ascorbic acid
Mdy Gin Aq
Linear (Ascorbic acid)
Linear (Mdy Gin Aq)
Ascorbic acid
y = 34.71x + 57.95
R² = 0.851
IC50 = 0.59 ug/ml
Mdy Gin Aq
y = 2.551x + 54.24
R² = 0.947
IC50 = 0.022 ug/ml


Mdy Gin EtOH
% Inhibition
log concentration ug/ml
Ascorbic acid
Mdy Gin EtOH
Linear (Ascorbic acid)
Linear (Mdy Gin EtOH)
Ascorbic acid
y = 34.71x + 57.95
R² = 0.851
IC50 = 0.59 ug/ml
Mdy Gin EtOH
y = 4.189x + 54.51
R² = 0.907
IC50 = 0.084 ug/ml


POL Gin Aq
% Inhibition
log concentration ug/ml
Ascorbic acid
POL Gin Aq
Linear (Ascorbic acid)
Linear (POL Gin Aq)
Ascorbic acid
y = 34.71x + 57.95
R² = 0.851
IC50 = 0.59 ug/ml
POL Gin Aq
y = 3.278x + 52.66
R² = 0.874
IC50 = 0.154 ug/ml


POL Gin EtOH
% Inhibition
log concentration ug/ml
Ascorbic acid
POL Gin EtOH
Linear (Ascorbic acid)
Linear (POL Gin EtOH)
Ascorbic acid
y = 34.71x + 57.95
R² = 0.851
IC50 = 0.59 ug/ml
POL Gin EtOH
y = 4.162x + 51.99
R² = 0.822
IC50 = 0.333 ug/ml

Discussion

ginger contained alkaloids, α amino acid, carbohydrate, flavonoids, glycosides, reducing sugar, saponins and tannins

Alkaloids - large variety of organisms, plants and animals, almost uniformly invoke bitter taste
- good analgesic, antispasmodic, antibacterial, anti-inflammatory, anticancer & antioxidant activities

Flavonoids - polyphenolic comp^{ds} in human diet & found in plants
- antiallergic, antibacterial, anticancer, antidiabetic, antidiarrhea, anti-inflammatory, antioxidant and lower risk of heart disease

Glycosides - important roles in living organisms, anticancer, antidiabetic, purgative, treatment of congestive heart failure, cardiac arrhythmia & skin diseases

Saponins - antibacterial, anticancer, antifungal, antiprotozoal, hypolipidemic, hypocholesterlemetric and responsible for central nerveous system

Tannins - antimicrobial agents [1,31, 32,35,36,37,38,39]

Macromineral Contents

Calcium - contents of Mdy & POL - 605 ppm & 291 ppm, within maximum permissible limit, (MPL) of WHO, 1996 High 'Ca' - important in medicinal plants, its role in bones, teeth, muscles system and heart functions

Magnesium - contents of Mdy & POL - 40 ppm comparable with other studies

Sodium - contents of Mdy & POL - 85 ppm & 83 ppm within MPL, WHO, 1996

Potassium - contents of Mdy & POL - 730 ppm & 739 ppm more than MPL of WHO, 1996 but comparable with other studies

Macrominerals- permissible limit of Ajasa, 2004

Micromineral Contents

Copper - contents of Mdy & POL - 3 ppm & 2 ppm
essential micronutrient

Iron - contents of Mdy & POL - 168 ppm & 209 ppm
essential trace element of human body tissues

Manganese - contents of Mdy & POL - 145 ppm & 168 ppm

Zinc - contents of Mdy & POL -13 ppm & 11 ppm
most abundant essential nutrients

All microminerals - within MPL of vegetables
set by FAO/WHO, 2001

According to antibacterial activity

ethanolic extract of ginger - more effective than aqueous extract
comparable to the study reported by Nikolić M *et al.* (2014), MIC and MBC values of ethanolic extract of ginger from Serbia, were 0.0024 mg/ml and 0.625 mg/ml on *S. aureus* and 2.5 mg/ml and 20 mg/ml on *P. aeruginosa*
Auta *et al.* (2011) investigated *Z. officinale* has antibacterial activity and *P. aeruginosa* more susceptible than *E. coli* [12]
Patil VB and Pwar NB (2016), reported that *E. coli* was susceptible to crude extracts of *garlic and ginger in vitro* which means the plant has antibacterial property [43]
differences in antibacterial activity - due to microorganisms used, method of extractions, type of solvents, different geography, climate and habitat of plant sampless

According to the antioxidant activity

aqueous extract of ginger - more effective than ethanolic extract
more potent than well-known antioxidant ascorbic acid
Mdy ginger was more potent than POL ginger
comparable to the study reported by Panpatil V V, *et al* (2013), ascorbic acid and Ethanol: water (1:1) extract of ginger from India was 9.34 µg/ml and 52.33 µg/ml [13]
Ginger serve as natural rich antioxidant food
enhance the immune system against oxidative damage,
may be utilized as a potential source of therapeutic agent

Conclusions

Ginger has quite a number of minerals and chemical constituents

responsible for many pharmacological activities and accordance with the medicinal usage of literature review

present study provide scientific base line data

helpful for herbal medicine users

local practitioners

pharmaceutical industries

standardization & quality control of indigenous drug using ginger for different types of ailments

Further investigation in other scientific area will show more of its potentials

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THANK YOU