International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 687-693
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Antimicrobial Activity of Ginger (*Zingiber officinale* Roscoe) Found in Manipur

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Received: 12 Mar 2019 / Accepted: 19 Apr 2019 / Published online: 1 Jul 2019
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Abstract

Due to indiscriminate use of antibiotics, antibiotic resistance has become a very common phenomenon. As such there is a need to search for plant based bioactive compound that can be harvested and used sensitively against microbes. Ginger, the spice has rich constituents of metabolites and is widely known for its therapeutic properties. Gas chromatography mass spectrometry (GCMS) analysis of the methanolic (Me), ethanolic (Et), petroleum ether (Pt) and water (Wa) extract of ginger reveals the presence of Zingerone (10.19% - 25.98%), [6]-shogaol (9.13% - 22.35%); [6]-gingerol (3.52% - 9.48%) as the major components. Antimicrobial studies on the Me, Et, Pt, Wa ginger extract and zingerone were conducted using two bacteria each of gram positive (*Staphylococcus aureus*, *Enterococcus faecalis*), two gram negative (*Salmonella typhi, Escherichia coli*) and two clinical isolates (*Bacillus subtilis*, *Shigella flexnari*). The antimicrobial test was conducted by adopting agar well diffusion method and the results showed bactericidal activity to both gram positive and gram-negative bacteria (pure culture or clinical isolates) with greater efficiency on gram positive bacteria, thus indicate that ginger extract may have various pharmaceutical applications.

Keywords

Antimicrobial, ginger extract, zingerone, agar well diffusion.

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INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is a spice that is widely proclaimed as a medicinal herb besides being used as a food additive. It belongs to the family Zingiberaceae and propagated mainly through rhizome. It is a pan-tropical crop and considered to be originated from South East Asia (Rabindran and Babu, 2004). Like any other spices, it has a rich aroma because of the various bioactive components present in it. The important characteristic feature of ginger is its pungency which is mainly imparted by gingerol (Suekawa et al., 1984) compound present in fresh ginger. Through a retro-aldol reaction, cooking or drying transforms gingerol to zingerone (Zhang et al., 2012) and is a pharmacologically active component in dry ginger. Shogaol is also one of the dehydrated forms of gingerol (Ali et al., 2008; Shahin, 2012). The rhizome contains 1-4% essential oil and oleoresin. Oleoresin is a dark viscous thick material obtained by the extraction of ginger powder with volatile solvent and has rich aroma. The amount of oleoresin obtain depend on the nature of solvent

used and the extraction process. More than 60 active constituents of ginger have been reported and are broadly divided as volatile (mostly monoterpenoid hydrocarbons and sesquiterpene) and non-volatile components like gingerols, shogaols, zingerone, paradols etc. (Ahmad et al., 2015). Due to its rich phytochemical, it exhibits various health beneficial properties like antioxidant, anti-inflammatory, anti-diarrhoeic, antimicrobial, hyperglycemia, anti-pyretic, analgesic etc. and is used for the treatment of number of diseases like nausea, diarrhoea, colic, arthritis, heart conditions, flu- like symptoms, stomach upset and painful menstrual periods (Altman et al.,2001; Sibanda and Okoh ,2007; Hassan,2017) The ginger oil and its extract were found to be effective against various microbes *Escherichia coli, Proteus* species, *Staphylococcus* species, *Streptococci* species, *Salmonella* species, *Bacillus subtilis*, *Pseudomonas aeruginosa* (Gupta et al.2014; Shahnaz et al.,2015; Jagetia et al.2003; Ali et al. 2008; Chan et al.,2007) . Till present the bioactive component and the antimicrobial studies of ginger found in the North- east region of India (Manipur) has not been reported. Moreover, the active constituents of ginger may vary significantly between plant cultivars, region of its cultivation. Hence , the present paper aims to identify the bioactive component present in the rhizome of the Ginger (*Zingiber officinale* Roscoe) found in Manipur , using different extracting solvent like methanolic (Me), ethanolic (Et), petroleum ether (Pt) and water (Wa) and to have a comparative study of antibacterial activities of the above ginger extract along with zingerone as authentic sample (Zingerone≥96% pure from Sigma Aldrich, USA) in reference to the zone of inhibition by using agar well diffusion method .

MATERIALS AND METHODS

Plant materials

Ginger rhizomes were collected from different districts of Manipur (Tamenglong, Chandel, Senapati, Ukhrul, Churchandpur , Imphal east and Imphal west). Collection was done during the harvesting season (Jan-March) i.e. when the rhizome was fully matured.

Sample preparation

The collected rhizome was washed in running tap water to remove dirt and cut into small slices. It is then air dried at room temperature. By using electric blender, it is made into powdered form stored at 4°C for further analysis.

For making methanolic (Me), ethanolic (Et), petroleum ether (Pt) and water (Wa) extract of ginger, 50 g of the powdered ginger was subjected to soxhlet extraction using 150 ml of respective solvent separately at temperature above the boiling point of the respective solvent.

After evaporating and concentrating the extract, it was then weighed and stored in the refrigerator at 4°C for further analysis.

GCMS analysis

The bioactive composition of the Me, Et, Pt and Wa extract of the ginger rhizome was analysed by GC-MS-QP-2010 Plus Ultra (Shimadzu) with a capillary column of Rxi-5 Sil MS (30mX0.5 mm i.d. X 0.25 µm film thickness). The column oven temperature was maintained at 60 °C for 2 min and then 250 °C for 3 min and finally 280 °C for 15 min. 2µl of 50 mg/2 ml ginger extract was injected with split ratio 10.0 and the injection temperature was 260°C with total flow of 16.3ml/min. The identification of the substance was done by comparison of the compound mass spectrum available in WILEY8.LIB, NIST14.lib and comparison of mass spectrum and retention index available in literature.

Antimicrobial assay

For assaying the antibacterial property of the Me, Et, Pt, Wa extract of ginger, 150 mg of the extract prepared in respective solvent is dissolved in 1 ml of Dimethyl Sulphoxide (DMSO) obtaining a concentration of 150 mg/ml. From this by dilution 100 mg/ml and 50 mg/ml concentration is prepared. For making zingerone (standard sample) extract, 150 mg of zingerone is also dissolved in 1ml of DMSO.

Microbial susceptibility by ginger extracts using agar-well diffusion

The antimicrobial assay of ginger extract was performed by deep-well agar diffusion method using Mueller Hinton agar (MHA) as the culture media. The test bacterial culture, two bacteria each of gram positive (*Staphylococcus aureus* ATCC 25923, *Enterrococcus faecalis* ATCC 29213), two gram negative (*Salmonella typhii*, *Escherichia coli* ATCC 25992) and clinical isolates (*Bacillus subtilis*, gram positive and *Shigella flexnari*, gram negative bacteria) were procured from Regional Institute of Medical Sciences (RIMS), Imphal, Manipur and antimicrobial sensitivity test was done in the Bacteriology Lab. of RIMS, Hospital, Imphal.

From the above pure culture, subculture was done in 20 ml of nutrient broth. The bacteria were allowed to grow by incubating in oven for 24-48 hour at 37°C. From the broth culture, by using sterile loop, inoculation was done in the prepared Mueller Hinton agar (MHA) plates separately with each of the test bacterial culture and evenly spread on entire surface of each plate. The plates were then kept in oven for 24 hours at 37°C. After incubation, the agar was

carefully punched using 20 mm sterile cork borer and 5 well were made in already bacterial seeded agar plates. In every plate, 100µl each of different concentration of ginger extract, DMSO and 50µl of 2 mg/ml streptomycin is loaded. Before incubation for 24 hours at 37°C, it was allowed to remain undisturbed for 1 hour so as to prevent spillage and for proper diffusion. Streptomycin, a broad-spectrum antibiotic was used as a positive control and DMSO as negative control. The above process is carried out in triplicate in the same manner for all the Me, Et, Pt, Wa extract of ginger and zingerone.

Zone of inhibition

The sensitivity of the Me, Et, Pt, Wa extract of ginger and zingerone were tested against the test organisms which was indicated by the appearance of clear zone (zone of inhibition) around the well. Zone of inhibition of *Staphylococcus aureus* is shown in fig.1, *Enterococcus faecalis* in Fig.2, *Salmonella typhii* in fig.3, *Escherichia coli* in Fig.4, *Bacillus subtilis* in fig.5, and *Shigella flexnari* in fig.6. The diameter of the clear zone was measured by using Hi antibiotic zone scale from HIMEDIA.

RESULTS AND DISCUSSION

The bioactive component of the Me, Et, Pt and Wa extract of the ginger rhizome was analysed by GC-MS-QP-2010 Plus Ultra (Shimadzu) with a capillary column of Rxi-5 Sil MS (30mX0.5 mm i.d. X 0.25 µm film thickness). Among the different solvent extract of ginger, maximum number of compounds was detected in Pt extract (135 compound) followed by Et (121), Me (63) and Wa extract (54) in the GCMS analysis data. Of all the compound detected (GCMS analysis data), those compounds constituting 1%and more than 1% of the total compound present in the respective solvent extract are shown in table 1. Fifteen (15) compound were detected in Mt, twenty-five (25) in Et, twenty-six (26) in Pt and six (6) in wa extract. The maximum % of compound content in all the extract was zingerone (10.19 - 25.98%), shogaol (6-,8-,10-), gingerol (4-,6-) and its derivatives. In Wa extract, 25.98 % of the total compound detected was zingerone while the least was 10.19% in Pt extract. The total composition of gingerol and its derivatives constitute about 50.55% in Mt extract, 40.22% in Et extract, 44.32% in Pt extract and maximum of 66.2% in Wa extract. The high percentage content of zingerone and shogaol may be due to high temperature treatment occurred during GC analysis. The antimicrobial assay of ginger extract performed by deep-well agar diffusion method using Mueller Hinton agar (MHA) shows that the Me and Wa extract of ginger are effective against all the six bacteria (Table2). It produced different zone of inhibition on varying concentration (50,100,150mg/ml) against *Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis* as shown in Fig.7. However, Et and Pt extract does not show any inhibition on *Enterococcus faecalis, Escherichia coli* and *Shigella flexnari* (Fig.7). Pt extract is not effective against any gram-negative bacteria and one-gram positive bacteria (*Enterococcus faecalis*) as shown in table 2 and Fig.7.

Table 1: GCMS analysis data of Me, Et, Pt, Wa extract of ginger having composition of 1% and more than 1% of the total compound present are shown in the present table.

Sl.no.	Compound name	Methanol	Ethanol	Petroleum ether	Water
		%	%	%	%
1	Octanal	1.04	1.44	1.33	0.83
2	Decanal	1.04	3.17	2.48	0.31
3	Alpha-curcumene	3.41	3.32	1.35	-
4	Alpha-zingiberene	0.81	5.92	0.11	-
5	Alpha;farnesene	0.32	2.28	-	-
6	Beta-bisabolene	1.02	1.65	0.33	-
7	Beta-Sesquiphellandrene	1.78	3.95	0.68	-
8	Nerolidol	1.05	1.18	0.97	-
9	4-(1-Hydroxyallyl)-2-methoxyphenol	0.31	0.89	0.24	0.78
10	Zingerone	11.60	13.06	10.19	25.98
11	2-Naphthalenemethanol, decahydro-. alpha.,. alpha.,4a-trimethyl-8-methylene-,	1.29	1.10	1.24	-
12	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-	1.23	0.91	0.06	-
13	4-(1,5-Dimethylhex-4-enyl) cyclohex-2-enone	-	-	1.37	-
14	3,7,11-TRIMETHYLDODECA-6,10-DIEN-1-YN-3-OL	1.32	0.99	1.27	-
15	Spiro [4.5] decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	-	-	0.83	-

16	2,5,5,8A-Tetramethyl-4-Methylene-6,7,8,8A-Tetrahydro-4H,5H-Chromen-4A-YL Hydroperoxide	3.25	-	3.50	1.55
17	(-)-5- Oxatricyclo[8.2.0.0(4,6)]Dodecane,,12-Trimethyl-9-methylene-	-	1.51	0.15	-
18	2,10-Dodecadien-1-ol, 3,7,11-trimethyl-	-	-	1.47	-
19	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	-	-	0.74	-
20	Hexadecanoic acid	5.78	3.26	4.56	4.75
21	Petasitene	1.03	-	0.89	-
22	9,12-Octadecadienoic acid (Z, Z)-	0.74	1.22	1.79	0.84
23	Oleic Acid	2.81	0.58	-	1.61
24	Cis-Vaccenic acid	-	1.56	1.90	0.34
25	[6]-Isoshogaol	4.18	2.59	3.00	6.21
26	(6)-Shogaol	14.17	9.13	11.35	22.35
27	[4]-Gingerol	2.10	-	-	0.41
28	[6]-Gingerol	3.52	5.23	5.77	9.48
29	[8]-Isoshogaol	0.98	0.82	1.05	0.23
30	[8]-Shogaol	-	3.75	5.03	1.54
31	[6]-Gingerdiol 3,5-diacetate	5.74	-	-	-
32	[10]-Isoshogaol	1.86	1.25	1.51	-
33	[10]-Shogaol	4.53	4.39	5.17	-
34	[6]-Gingerdiol (2E)-geranial acetal	1.87	-	1.25	-
35	Stigmasterol	-	0.60	1.21	-
36	Gamma. -Sitosterol	2.59	-	3.83	-
37	Beta-sitosterol	-	2.36	-	2.40

*Compound that are present in the solvent extract but constitute less than 1% of the total compound present in the respective solvent extract.

Table 2: The observed Zone of inhibition of various bacteria by the Me, Et, Pt,Wa extract of ginger extract and Zn.

Test organism	Me extract (mg/ml)			Et extract (mg/ml)			Pt extract (mg/ml)			Wa extract (mg/ml)			Zn (≥96% pure)			Streptomycin (mg/ml)
	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50
Staphylococcus aureus	12±0	13.33 ±0.33	17.67 ±0.88	12±0	18.67 ±0.33	22±0	21.67 ±0.33	25 ±0.58	26.33 ±0.33	16 ±0.58	18.67 ±0.33	21 ±0.58	.... .	18.33 ±1.20	20.67 ±0.33	27 ±0.57
Enterrococcus faecalis	12±0	15.33 ±0.33	16.33 ±0.33	.....	.....	.....	.....	.....	.....	12±0	12.67 ±0.67	14 ±0.58	16 ±0	17 ±0	23 ±0	22
Salmonella typhii	11.33 ±0.33	14 ±0.58	15.67 ±0.88	.....	12.33 ±0.33	13.33 ±0.33	.....	....	.....	.....	12±0	13±0	.... .	16.33 ±1.20	18 ±0.58	22.67 ±0.67
Escherichia coli	.....	14.33 ±0.33	15.33 ±0.33	.....	.....	.....	.....	.....	.....	12.33 ±0.67	15.33 ±0.33	16.33 ±0.33	.... .	15.67 ±0.33	18.67 ±0.33	24 ±1.15
Bacillus subtilis	14.33 ±0.33	16.33 ±0.33	18.33 ±0.33	15.33 ±0.33	19.33 ±0.33	22.33 ±0.33	19.33 ±0.33	21 ±0.33	23 ±0.58	14.3 3±0.33	18.33 ±0.33	20.33 ±0.33	.... .	14±0	17±0	24.67 ±0.67
Shigella flexnari	.....	13.33 ±0.33	16.33 ±0.33	.....	.....	.....	.....	.....	.....	12±0	15.33 ±0.33	16.33 ±0.33	.... .	.....	.....	.....

While in our study, Wa extract is found to be effective against both the gram +ve and gram –ve bacterium, Et extract against only *Bacillus subtilis*. But Akintobi et al., 2013, reported that ginger extract of ethanol and water was ineffective against *Escherichia coli* and *Bacillus subtilis*.

When the zingerone pure is subjected to antibacterial testing of the above bacteria, it shows highest inhibition against gram positive bacteria (*E. faecal* – 23 ± 0 mm, *Staphylococcus aureus* - 20.67 ± 0.33mm, *Bacillus subtilis* – 17 ± 0 mm) than on gram negative bacteria (*Salmonella typhii* – 18 ± 0.58 mm , *Escherichia coli*- 18.67±0.33mm, *Shigella flexnari*- 0mm as shown in Table2.) as reflected by effective amount of concentration and size of inhibition. At 50mg/ml when zingerone does not show any inhibition on growth of bacteria like *Staphylococcus aureus, Salmonella typhii, Escherichia coli* and *Bacillus subtilis*, Me and Wa extract show inhibition. Wa and Me extract contain more percentage of gingerol derivatives (66.2% and 50.55% respectively) compared to Pt (44.32%) and Et extract (40.22%).

In Pt and Et extract, where gingerol derivatives (and zingerone) are present in comparatively lesser

quantity, less antibacterial activity is observed although presence of higher number of compounds are detected in these extracts in GCMS analysis. Pt extract is found ineffective against any gram-negative bacteria.

Even though all the different extract of ginger and zingerone show wide range of antibacterial activity against both the gram positive and gram-negative bacteria, highest zone of inhibition is observed in gram positive bacteria. Similarly Kaushik and Goyal (2011), Hassan et al. (2012), also observed that the methanol extract of ginger was more effective against the gram positive bacteria compared to gram negative bacteria and he also reported that methanol extract was more effective against Gram negative organism *Pseudomonas aeruginosa* than the gram positive bacteria like *Bacillus subtilis.*

Fig.1: Zone of inhibition shown by different ginger extract-Me, Et, Pt, Wa and Zn against S.aureus

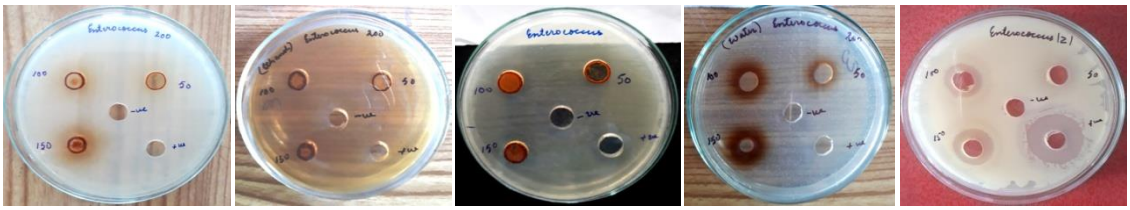

Fig.2: Zone of inhibition shown by different ginger extract-Me, Et, Pt, Wa and Zn against E.faecalis

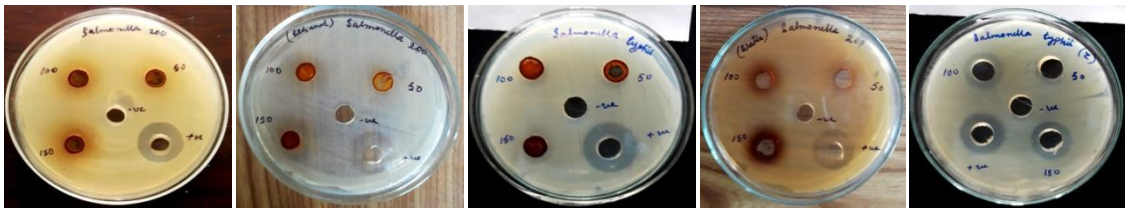

Fig.3: Zone of inhibition shown by different ginger extract-Me, Et, Pt, Wa and Zn against S.typhii

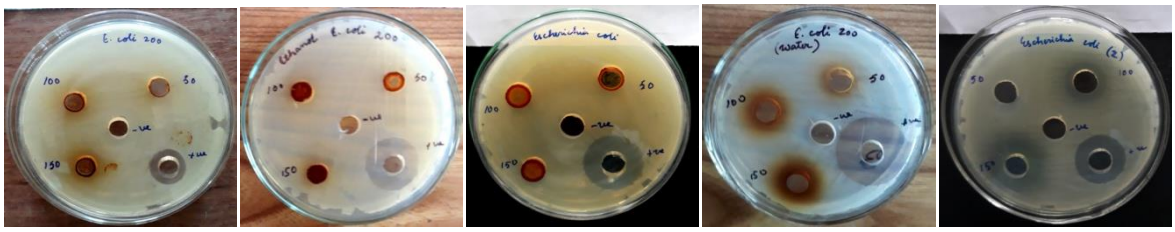

Fig.4: Zone of inhibition shown by different ginger extract-Me, Et, Pt, Wa and Zn against E.coli

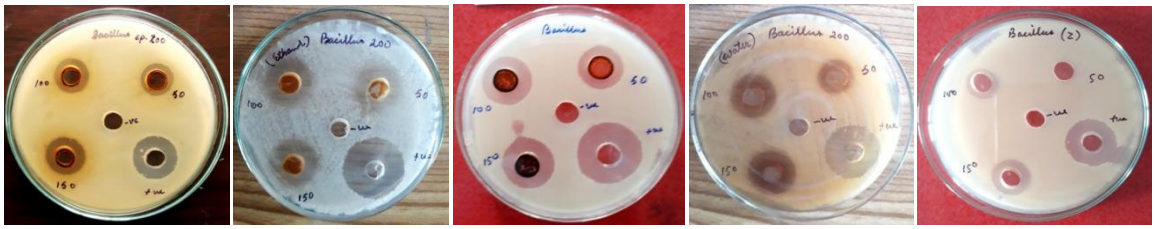

Fig.5: Zone of inhibition shown by different ginger extract-Me, Et, Pt, Wa and Zn against B.subtilis

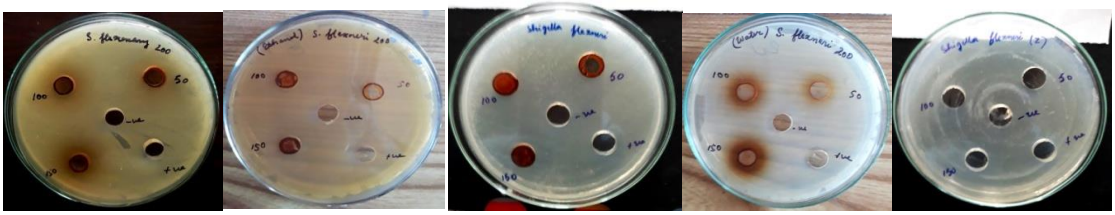

Fig.6: Zone of inhibition shown by different ginger extract-Me, Et, Pt, Wa and Zn against S.flexnari

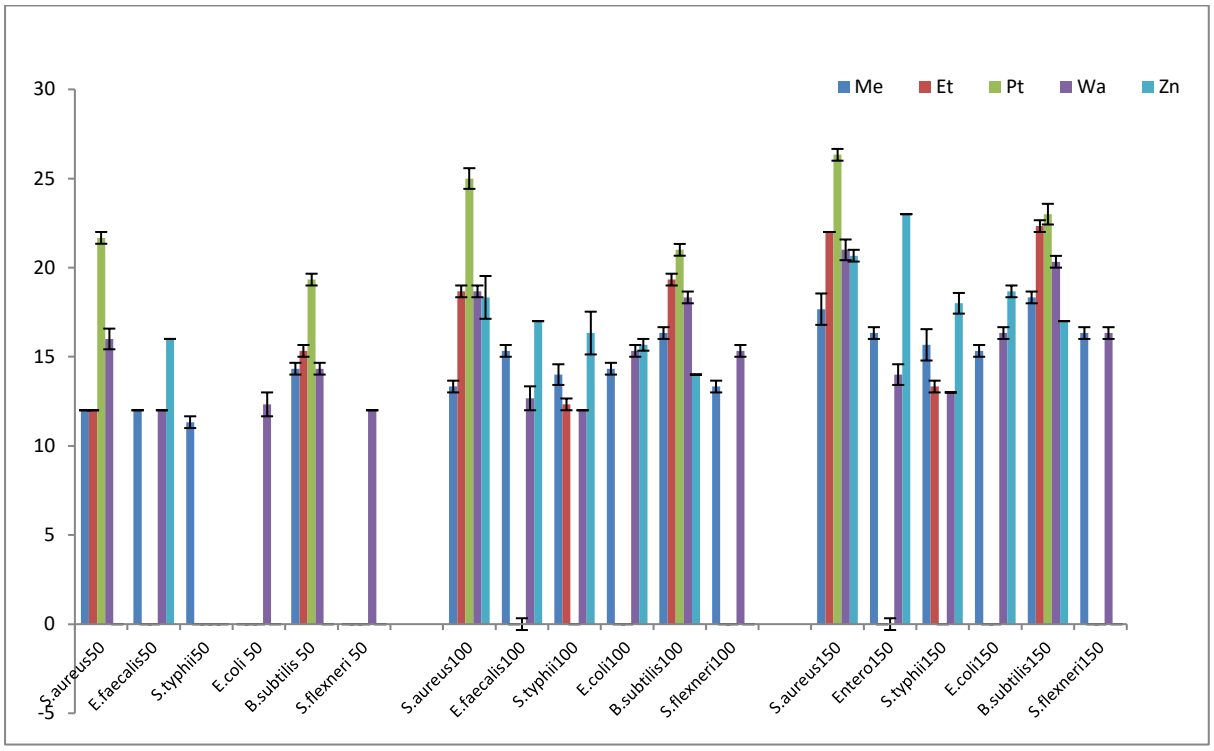

Fig.7: Histogram of Table 2
All the data are depicted in the form of Mean ± Standard error

CONCLUSIONS

Irrespective of the nature of the solvent used for the extraction process, high temperature treatment, ginger extract shows antibacterial activity to both gram positive and gram-negative bacteria (pure culture or clinical isolates) with greater efficiency on gram positive bacteria. The GCMS analysis of the different solvent ginger extract indicated presence of compound belongings to steroids, aldehyde, phenol, sesquiterpene etc. But the phenolic and ketonic compounds like gingerol, shogaol and zingerone were found in high percentage. The antibacterial property of the ginger is attributed to the presence of these compound and synergistic effect of other compound like alpha-curcumene, beta-sesquiphellandrene, alpha-zingiberene, hexadaeconic acid, oleic acid etc. This justifies the usefulness of adding ginger in everyday cooking especially in South East Asia and the uses of ginger in various forms like pickles, salad, bread, candies or as mouth freshener. Now it is the need of the hour to identify, isolate and to work out the underlying mechanism to maximise the efficiency of the bioactive compound present in ginger for it to find wider application in pharmaceutical and food industry. This is of great significance when the antibiotic resistance has increased randomly, and the global problem of antibiotic resistance has led to the isolation and characterisation of new antimicrobial compounds in the plant products.

ACKNOWLEDGEMENTS

This research has been supported by UGC-CSIR, New Delhi, Govt. of India in the form of Junior Research Fellowship to the first author

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