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Evaluation of Antibacterial Activity and GCMS Analysis of *Zanthoxylum Ovalifolium* Fruit Extracts

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Authors' contributions

This work was carried out in collaboration between both authors. Author PP conducted the experiment, wrote the protocol, recorded the data, performed the statistical analysis, conduct the experiment and author RN planned the experiment, analyzed the study, monitored the experiment and drafted the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Herbal medicines have played a vital role in the evolution of humanity and civilization. The fruits of the plant *Zanthoxylum* were collected from the study field, washed, dried in the shade and powdered. Soxhlation method was used to make different solvent extract of fruit. The different fruit extracts were examined against *P. aeruginosa, P. syringe, S. aureus, S. typhi, K. pneumonia* and *E. coli* by agar well diffusion method and for the detection of biochemical components present in the *Zanthoxylum* fruit, methanolic fruit extract was analyzed using GC-MS. Results showed that, the methanolic extract of fruit is found to be exhibit activity against P. aeruginosa, P. syringe, S. aureus, S. typhi, K. pneumonia and E. coli when compared to the hexane and ethyl acetate. The discovery of 46 bioactive compounds in methanolic fruit extract was verified by their molecular formula, molecular weight, and area peak percentage. In which 6 bioactive compounds such as 6-octadecanoic acid, Pentadecanoic acid, Ethyl oleate, 9-octadecenoic acid (Z)-, methyl ester, Glycerin and 1, 2-dimethoxy-4-(2-methoxyethenyl) benzene compounds showed highest peak area percentage of 26.14, 13.61, 8.56, 6.12 and 6.08% respectively. The compounds found in this research may be responsible for some of the medicinal action of the *Z. ovalifolium* fruit and may be useful in the development of new medicines.

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Keywords: Zanthoxylum ovalifolium; fruit extracts; Pentadecanoic acid; Herbal medicines; antibacterial activity.

1. INTRODUCTION

The value of medicinal herbs was discovered as early as the middle Ages. There were no synthetic drugs available at the moment, so they depended entirely on natural medicines to cure all illnesses. As a result, we can deduce that plants have a wide variety of medicinal properties and are highly helpful to human health and well-being. Biological research is needed to learn more about the healing uses [1].The therapeutic benefit of such noteworthy plants consists in certain chemical compounds that give the human body a definite physiological effect. T he most significant aspect of these bioactive plant components is that, they can be used as chemotherapeutic compounds, in effective curing of different ailments in human being and also safer to use without side effects. But in case of synthetic drugs, there may be chances of side effects, hypersensitivity and allergic reactions in treated patients [2].

WHO (World health organization) reported that, in developing countries about 80% of the population still rely on traditional system medicine for their medicinal purposes needs mainly plant drug. Unani, Ayurveda, Homeopathy, and Siddha accounted for about 95% of all prescriptions in India. The medicine practitioners used local known medicinal plants in traditional way to tread various ailments [3].

GC-MS is a hyphenated approach that is the most widely used technique for identification and quantification. Through interpreting the spectra and comparing them to reference spectra, the unknown organic compounds in a can be calculated [4].

The plant *Zanthoxylum ovalifolium* belongs to family Rutaceae (citrus family), many medicinal plants of this *Zanthoxylum* genus showed pharmacological such as antibacterial, antifungal, analgesic, anti inflammation due to the presence of bioactive compounds in it [5]. *Z. ovalifolium* fruit have several essential phytochemical compounds such as alkaloids, flavonoids, steroids, tannins, and phenol [6-7], which must be intensely evaluated for the isolation of effective bioactive compound(s) in curing different ailments. The aim of this research is to use GC-MS to identify bioactive chemicals in methanolic leaf extract of *Z. ovalifolium* and also evaluating the antibacterial activity of different extract.

2. METHODOLOGY

2.1 Collection of Plant Material

From March to August, plant fruits were collected from the study area's plains and mountains. In certain cases, local people's interactions have been used to decide the best site for plants. We tried to use conventional methods. The dried fruits of *Z. ovalifolium* were used as a source of plant material for the current study. The fruit was harvested from the Sringeri forest in the Chikkamagaluru district of Karnataka. The fruits of *Z.ovalifolium* were taxonomically classified using normal flora (1, 2, and 3). The herbarium specimen was stored and voucher specimen number KU/AB/RN/PP/002/2017 was placed in the dept. of Botany, Kuvempu University herbarium collection.

2.2 Preparation of Plant Sample

The Fresh plant material fruits of *Z. ovalifolium* were collected from the study area and separated, washed with running water for about 2-3 times and allowed for 20- 30 days of shade dried. These dried plant samples were pulverized to the coarse powder of about 1mm in diameter using a mechanical grinder. The powdered materials were kept for 4°C and used to for further study [8-11]

2.3 Preparation of Extracts

In this study, different solvents was used to separate the components of *Z. ovalifolium* fruits based on i ncreasing in the polarity of solvents. Extraction of *Z. ovalifolium* carried out using a Soxhlet apparatus. E qual amount (250) of powdered fruit sample are extracted using different solvents (Hexane, ethyl acetate a nd methanol) and extraction process continued till the solvent present in the siphon tube turns to colorless then concentrated using rotary evaporator and extracts kept at 4°C until used [12-15].

Image 1. A- plant fruit **B- shade drying of fruit**

2.4 GC-MS Analysis of Plant Extract

Quantitative GC-MS analysis of fruit extracts of *Z. ovalifolium* was done IADFAC LABORATORIES PVT LTD, Bangalore. Methanolic leaf and fruit extract of plant were subject to GC-MS (gas chromatography and mass spectroscopy) to identify the bioactive compounds present in plant sample [13].

2.5 Selection of Bacterial Strains

Antibacterial activities of the fruit extracts of *Z. ovalifolium* were screened against clinical isolated Gram- positive and Gram-negative pathogen bacterial strains were stored in department of Applied Botany, Kuvempu University, shankaraghatta. On nutrient broth at 37°C, pathogenic bacteria were maintained.

2.6 Agar Well Radical Diffusion Assay

The antibacterial activity of *Z. ovalifolium* fruit extracts was evaluated by using standard agar well radial diffusion method against selected pathogenic bacteria. The sterilized nutrient agar medium was poured into sterilized glass petri plates. Liquid broth containing 100 µl of 24 h previous bacterial cultures was spread separately over the solid nutrient agar media plates and it was punched by using sterilized cork borer of size about 6mm diameter. Each well was loaded with 25 µL leaf extract of different solvent (Hexane, Ethyl acetate Methanol) and concentration like, 25, 50, 100 µg/ml and negative control (DMSO) and standard drug 1µg/ml .The culture plates were incubated at 37°C for 24 h. the experiment was triplicate for each extract.

3. RESULTS

3.1 Extract Yield

Due to less extract yield and less confirmation of phytochemicals in hexane and ethyl a cetate extract (1.25 grams and 2.13 grams) we have conducted only antimicrobial activity for these two e xtracts (hexane and ethyl acetate) and only methanolic extract (12.46 grams) is subjected for GCMS as w ell as antimicrobial studies.

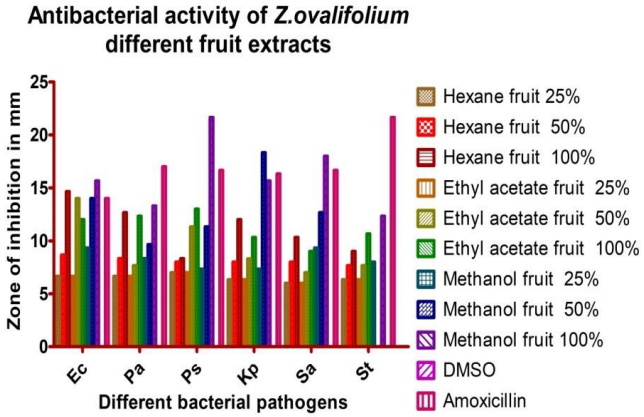

Fig. 1. Antibacterial activity of different fruit extract of *Z. ovalifolium*

Table 1. Antibacterial activity of different fruit extract of *Z. ovalifolium*

	Hexane 25	Hexane 50	Hexane 100	Ethyl acetate 25	Ethyl acetate 50	Ethyl acetate 100	Methanol 25	Methanol 50	Methanol 100	DMSO	Amoxicillin
Ec	6.66±1.15	8.66±1.52	14.66±1.15	6.66±1.15	14±1	12±1	9.33±1.54	14±1.3	15.66±1.15	0±0	20.66±1.73
Pa	6.66±1.15	8.33±1.52	12.66±1.15	6.66±1.15	7.66±1.55	12.33±0.57	8.33±0.57	9.66±0.57	13.3±1.52	0±0	17±1
Ps	7±1.15	8±0.57	8.33±0.57	7±1.15	11.33±0.57	13±1	7.33±0.57	11.33±0.57	21.66±1.52	0±0	16.66±1.54
Kp	6.33±2.3	8±2	12±1.73	6.33±2.3	8.3±1.54	10.33±1.57	7.33±1.57	18.33±2.08	15.66±1.52	0±0	16.33±1.52
Sa	6±1	6±1	10.33±1.52	6±1	6±0	9±1.73	9.33±2.88	12.66±1.15	18±1.73	0±0	18.6±1.1
St	6.33±1.52	6.66±1.52	8±1.73	6.33±1.52	7.66±0.57	10.66±1.52	8±0	9±1	12.33±1.15	0±0	21.66±1.15

***Ec**: Escherichia coli, Pa: Pseudomonas aeruginosa, Ps: Pseudomonas syringae, Kp: Klebsiella pneumonia, Sa: Staphylococcus aureus,St: Salmonella typhi*

Table 2. Phytoconstituents identified in methanolic fruit extract of *Z. ovalifolium* showed biological activity

Sl.no	Compound	Area peak%	MF	MW	Biological activity
1	2-cyclopenten-1-one,2- hydroxy	0.67	$C_5H_6O_2$	98	Antimicrobial, Anti-inflammatory, Anticancer, Dieruretic. [7]
2	Glycerin	6.08	$C_3H_8O_3$	92	Preservative antimicrobial[8]
3	1,2,3- propanetriol, monoacetate	0.64	$C_5H_{10}O_4$	134	Antimicrobial [9]
4	Benzofuran,2,3-dihydro	0.21	C_8H_8O	120	Antimicrobial, Anti-inflammatory[7]
5	2(R),3(S)-1,2,3,4-butanetetrol	1.01	$C_4H_{10}O_4$	122	Biological activity, Biosurfactants[10]
6	2-methoxy-4-vinylphenol	0.22	$C_9H_{10}O_2$	150	Analgesic and anti-germination [11]
7	Benzene,1,2,5- trimethoxy-	1.07	$C_9H_{12}O_3$	168	Unknown
8	Phenol,3,5-dimethoxy-	0.77	$C_8H_{10}O_3$	154	Unknown
9	Naphthalene,1,2,3,4,4a,5, 6,8a-octahydro-7-methyl- 4-methylene-1-(1-methylel)-,(1.a)	0.75	$C_{15}H_{24}$	204	Unknown
10	1H-cyclopro[e]azulen-7- ol,decahydro-1,1,7- trimethyl-4-methylene-1- (1ar-(1a.alpha.,4a)	1.03	$C_{15}H_{24}O$	220	Unknown
11	1,2-dimethoxy-4-(2-methoxyethenyl)benzene	4.80	$C_{11}H_{14}O_3$	194	Flavoring Compounds
12	(1R,3R,4R,5R)-(-)-Quinic acid	2.84	$C_7H_{12}O_6$	192	Starting material for synthesis of medicine for treatment of influenza A and B [12]
13	10,12-Docasadiyndioic acid	0.26	$C_{22}H_{34}O_4$	362	Unknown

Sl.no	Compound	Area peak%	MF	MW	Biological activity
14	Benzaldehyde,2-hydroxy- 6-methyl	0.31	$C_8H_8O_2$	136	Used in the treatment Cancer, sexual or genital disorder, antipyretic, anti-inflammatory, analgesic, treatment in immunological or allergic disorder [13]
15	DL-Tryptophan	0.27	$C_{11}H_{12}N_2O_2$	204	Unknown
16	N,N-Dimethyltryptamine	1.54	$C_{12}H_{16}N_2$	188	Insecticide and antibacterial [14]
17	Hexadecanoic acid, methyl ester	2.52	$C_{17}H_{34}O_2$	270	Antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, hemolytic, 5-Alpha reductase inhibitor [15]
18	Pentadecanoic acid	13.61	$C_{15}H_{30}O_2$	242	Antimicrobial[15]
19	Hexadecanoic acid, methyl ester	1.60	$C_{18}H_{36}O_2$	282	Antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, hemolytic, 5-Alpha reductase inhibitor [15]
20	Eicosanoic acid	0.44	$C_{20}H_{40}O_2$	312	Antibacterial, antifungal, antioxidant [16]
21	9,9-domethoxybicyclo[3.3.1] nona-2,4-dione	0.56	$C_{11}H_{16}O_4$	212	Unknown
22	9-octadecenoic acid (Z)-, methyl ester	6.12	$C_{19}H_{36}O_2$	296	Anti-inflammatory, Anti-androgenic Cancer preventive, Dermatitigenic, Hypocholesterolemic, 5- Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor[17]
23	Octadecanoic acid (Z)-, methyl ester	0.97	$C_{19}H_{38}O_2$	298	Antimicrobial [18]
24	6-octadecanoic acid	26.14	$C_{18}H_{34}O_2$	282	Cancer preventive, Insectifuge [19]
25	Eicosanoic acid	0.42	$C_{20}H_{38}O_2$	310	Antibacterial, antifungal, antioxidant [16]
26	Ethyl oleate	8.56	$C_{20}H_{40}O_2$	312	Flavoring compounds
27	Octadecanoic acid, ethyle ester	0.42	$C_{17}H_{32}O$	252	No activity reported
28	8- hexadecanal, 14- methyl-,(Z)-	0.35	$C_{20}H_{40}O_2$	312	Not found
29	2-(2-methyl-3-oxocyclohexyl)thiopropio nic acid,S-t-butyl ester	0.35	$C_{14}H_{24}O_2S$	256	Unknown
30	Eicosane	0.40	$C_{20}H_{42}$	282	Antibacterial, antifungal, antioxidant [16]
31	Tetratriaconntane	0.28	$C_{34}H_{70}$	478	Plant growth promotion [20]
32	Hexadecanoic acid,2- hydroxy-1-(hydroxymethyl)ethyl ester	0.53	$C_{19}H_{38}O_4$	330	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor[21]
33	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	1.65	$C_{19}H_{34}O_2$	294	Antimicrobial activity [22]
34	Phenol,3-[(trimethylsilyl)oxy]	0.24	$C_9H_{14}O_2Si$	184	Unknown

Sl.no	Compound	Area peak%	MF	MW	Biological activity
35	Heptacosane	0.22	C27H56	380	Antifungal [20]
36	(p-Decanoylphenoxy)acetic acid	1.85	C18H26O4	306	Unknown
37	Acetamide, 2-(3,4-dimethoxyphenyl)-N-(9- oxo-9H-fluoren-2-yl)-	0.63	C23H19NO4	373	Unknown
38	Mescalin-propionyl	0.36	C14H21NO4	267	Unknown
39	Cholest-5-en-3-ol (3.beta.)-	0.62	C14H21NO4	386	Antibacterial and anti inflammatory activity[15]
40	Ergost-5-en-3-ol, (3.beta.)-	1.74	C28H48O	400	Antimicrobial, anti-inflammatory effects [15]
41	Stigmasterol	1.01	C29H48O	412	Antihepatotoxic, Antiviral, Antioxidant, Cancer preventive, Hypocholesterolemi[14], anti- microbial [23]
42	gamma.-Sitosterol	3.54	$C_{29}H_{50}O$	414	Plant and animal growth hormone, anti-ulcer, antimicrobial, antipyretic anti-inflammatory, antidiarrhoeal and hypolipidemic agent[24][25]
43	5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro- 3,8,8,11a-tetramethyl-, [3S-(3.alpha.,5	0.77	$C_{18}H_{30}O_2$	278	Fragrance agent
44	Stigmasta-3,5-dien-7-one	0.40	$C_{29}H_{46}O$	410	Unknown
45	Stigmast-4-en-3-one	0.94	$C_{29}H_{48}O$	412	Antitumour, hypoglycaemic and antidiabetic [26]
46	Cholestan-3-one, 4, 4-dimethyl-, (5.alpha.)	0.28	$C_{29}H_{50}O$	414	Unknown

MW- molecular weight, MF- molecular formula

3.2 Antibacterial Activity

The result revealed that *Z. ovalifolium* fruit of varies solvent extract (hexane, ethyl acetate and methanol solvent) were showed appreciable antibacterial activity by suppressing the growth of tested human pathogenic as well as plant pathogenic bacteria specimens with the standard drug antibiotic Amoxicillin at varying concentrated doses of 25mg/ml, 50mg/ml and 100mg/ml.

Hexane leaf extract showed significant amount of zone of inhibition against bacterial strains at different
dose levels (25mg/ml, 50mg/ml 100mg/ml) at 100 mg/ml of concentrate showed different zone of inhibition against bacterial strains viz., Escherichia *coli* (14.66±1.15), *Pseudomonas aeruginosa* (12.66±1.15), *Klebsiella pneumonia* (12±1.73), *Staphylococcus aurous* (10.33±1.52), *Pseudomonas syringae* (8.33±0.57) and *Salmonella typhi* (8±1.73) (Table 1; Fig. 1)

In the same way in ethyl acetate extract at 100mg/ml dose level showed significant suppression of bacterial growth against *Pseudomonas syringae* (13±1), followed by *Pseudomonas aeruginosa* (12.33±0.57),*Escherichia coli* (12±1), *Salmonella typhi* (10.66±1.52), moderate activity against *Klebsiella pneumonia* (10.33±1.57), and least zone of inhibition was found in *Staphylococcus aureus* (9±1.73). (Table 1; Fig. 1)

While, in methanolic extract i.e at 100mg/ml showed significant activity against pathogenic bacteria i.e,the highest zone of inhibition was found against Pseudomonas *syringae* (21.66±1.52), followed by *Staphylococcus aureus* (18±1.73), *Klebsiella pneumonia* (15.66±1.52), *Escherichia coli* (13.66±1.15), *Pseudomonas aeruginosa* (12.3±1.52) and *Salmonella typhi* (12.33±1.15). (Table 1; Fig. 1)

3.3 GCMS analysis

GC-MS analysis of methanolic fruit extract of *Z. ovalifolium,* confirmed the presence of 46 compounds with their molecular formula, molecular weight and area peak percentage. Out of which, 31 compounds were known for its biological properties, where, 7 compounds were antimicrobial property, 4 compounds were antibacterial compounds, 4 compounds were antifungal activity, 5 compounds were anti nflammatory compounds, 9 compounds of antioxidant compounds, 4 compounds were anticancer compounds, 7 flavoring ompounds, 2 compounds were analgesic, rest of compounds were anti-diabetic, anti depression, anti-germination, anti- androgenic, plant growth, antihepatotoxic and antidiuretic. Remain 15 compounds biological properties are not found (Table 2; Fig. 2).

The known compound of antimicrobial compounds present in fruit of *Z.ovalifolium* are, 2-cyclopenten-1- one,2-hydroxy (0.67 %), Glycerin (6.08%), 1,2,3- propanetriol, monoacetate(0.64%), Benzofuran,2,3-dihydro (0.21%), Eicosanoic acid (0.44 %), Octadecanoic acid (Z)-, methyl ester (0.97 %), Eicosane (0 .40 %), E,E,Z-1,3,12-Nonadecatriene-5,14-diol(1.65 %), Heptacosane (0.22%), Cholest-5-en-3-ol (3.beta.)- (0.62%), Stigmasterol (1.01 %) and gamma.-Sitosterol (3.54 %). (Table 2; Fig. 2)

The known anti-inflammatory compounds present in *Z.ovalifolium* fruit were, 2-cyclopenten-1-one,2- hydroxy (0.67 %), Benzofuran,2,3-dihydro (0.21%), 9-octadecenoic acid (Z)-, methyl ester (6.12 %) and Cholest-5-en-3-ol (3.beta.)- (0.62%). (Table 2; Fig. 2)

The GC-MS reveals the presence known anti-cancer compounds present in *Z.ovalifolium* fruit were, 2-cyclopenten-1-one,2-hydroxy (0.67 %), Benzaldehyde,2-hydroxy-6-methyl (0.31%), 9-octadecenoic acid (Z)-, methyl ester (6.12%), Stigmasterol (1.01 %) and Stigmast-4-en-3-one (0.94%). (Table 2; Fig. 2)

The analgesic property present in *Z.ovalifolium* fruit were, 2-methoxy-4-vinylphenol (0.22 %), 2-methoxy-4-vinylphenol (0.22 %) and Benzaldehyde,2-hydroxy-6-methyl (0.31%). (Table 2; Fig. 2)

The *Zanthoxylum ovalifolium* fruit with anti-oxidant properties were, Hexadecanoic acid, methyl ester (2.52 %), Hexadecanoic acid, methyl ester (1.60 %), Eicosanoic acid (0.44 %), Eicosanoic acid (0.42 %), Eicosane (0.40%), Ergost-5-en-3-ol, (3.beta.)- (1.74%), Stigmasterol (1.01 %) and .gamma.-Sitosterol (3.54%). The identified compounds of antidepressant, antidiuretic, antigermination, Antihepatotoxic, antiviral, antidiabetic, insectifuge and pesticides compounds were, N,N-Dimethyltryptamine (1.54%), 2-cyclopenten-1-one,2-hydroxy (0.67%), 2-methoxy-4-vinylphenol (0.22%), Stigma sterol (1.01%), S

tigmast-4-en-3-one (0.94 %), 9-octadecenoic acid (Z)-, methyl ester (6.12 %) and Hexadecanoic acid,2-h ydroxy-1-(hydroxymethyl)ethyl ester(0.53%). (Table 2; Fig. 2)

The identified flavoring compounds present in *Zanthoxylum ovalifolium* were, 1,2-dimethoxy-4-(2-methoxyethenyl)benzene (4.80%),Ethyl oleate (8.56%),Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester (0.53%), 5H-3,5a-Epoxynaphth[2,1-c]oxepin and dodecahydro-3,8,8,11a- t etramethyl-, [3S-(3.alpha.,5 (0.77%). (Table 2; Fig. 2)

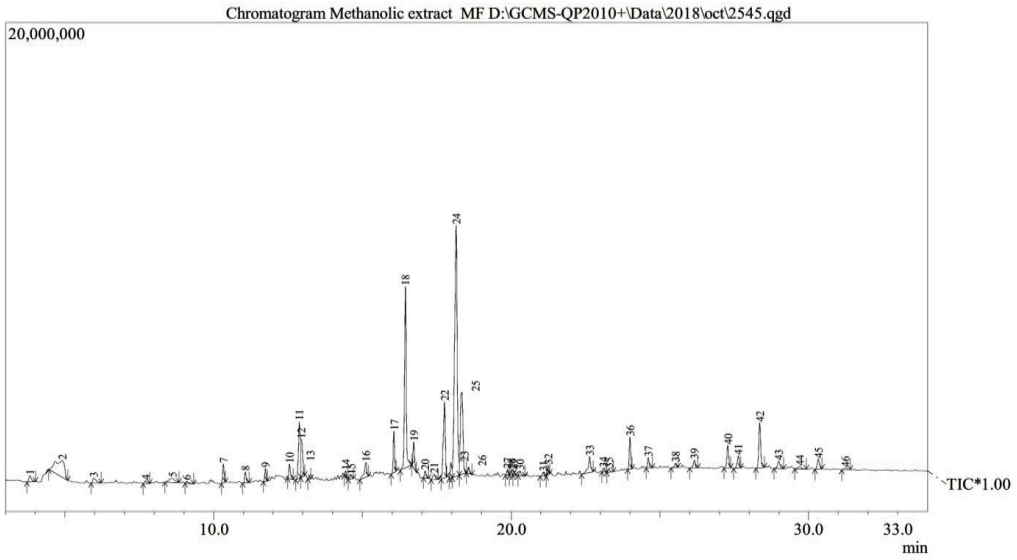

Fig. 2. GC-MS Chromatogram of methanolic fruit extract of *Z.ovalifolium*

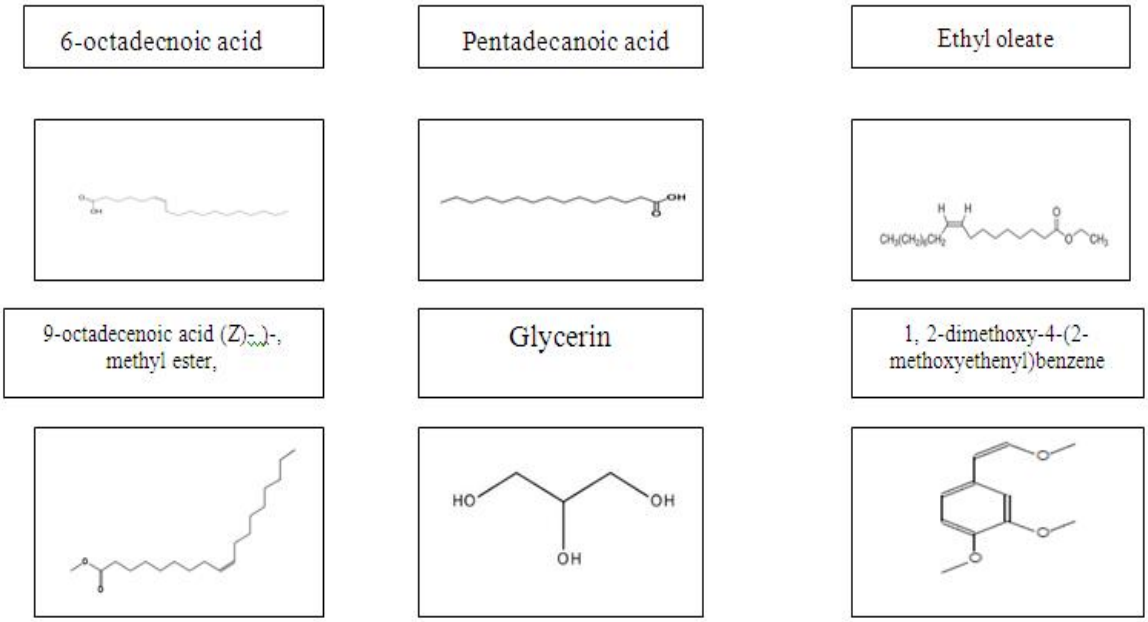

Fig. 3. Characterized compound structure from methanolic fruit extract of *Z. ovalifoliu*

4. DISCUSSION

The result revealed that *Z. ovalifolium* fruit of varies solvent extract (hexane, ethyl acetate and methanol solvent) were showed appreciable antibacterial activity by suppressing the growth of tested human pathogenic as well as plant pathogenic bacteria specimens with the standard drug antibiotic Amoxicillin at varying concentrated doses of 25mg/ml, 50mg/ml and 100mg/ml.

When compared with hexane and ethyl acetate extracts, the methanolic extract showed optimum zone of inhibition and the best activity was observed against Pseudomonas *syringae, Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* (Table1; Fig.1).

Whereas, at a concentration of 10 mg/ml, the standard antibiotic amoxicillin treated against pathogenic bacteria exhibit a higher rate of inhibition zone against the pathogenic bacteria tested. The degree of zone of inhibition was found in the following order i.e., *Salmonella typhi* > *Escherichia coli* > *Staphylococcus aureus* > *Pseudomonas aeruginosa*> *Pseudomonas syringae* > *Klebsiella pneumonia.* (Table1; Fig. 1).

GC-MS analysis of methanolic fruit extract of *Z. ovalifolium,* confirmed the presence of 46 compounds w ith their molecular formula, molecular weight and area peak percentage. Out of which, 31 compounds w ere known for its biological properties, where, 7 compounds were antimicrobial property, 4 compounds w ere antibacterial compounds, 4 compounds were antifungal activity, 5 compounds were antinflammatory compounds, 9 compounds of antioxidant compounds, 4 compounds were anticancer compounds, 7 flavoring compounds, 2 compounds were analgesic, rest of compounds were anti-diabetic, anti depression, anti-germination, anti- androgenic, plant growth, antihepatotoxic and antidiuretic. R emain 15 compounds biological properties yet to be explored (Table 2; Fig. 2).

T he six major peaks reflecting six major compounds were described and categorized by comparing the mass spectra of the constituents with compound library such as, 6-octadecanoic acid, Pentadecanoic acid, E thyl oleate, 9-octadecenoic acid (Z)-, methyl ester, Glycerin and 1, 2-dimethoxy-4-(2-methoxyethenyl) b enzene compounds were major category of compounds mainly confirmed in the fruit extract (Fig. 3).

T he known compound of antimicrobial agent present in fruit of *Z.ovalifolium* are, 2-cyclopenten-1-one,2-hydroxy (0.67 %), Glycerin (6.08%), 1,2,3- propanetriol, monoacetate(0.64%), Benzofuran,2,3-dihydro (0.21%), Eicosanoic acid (0.44 %), Octadecanoic acid (Z)-, methyl ester (0.97 %), Eicosane (0.40 %), E,E,Z-1,3,12-Nonadecatriene-5,14-diol(1.65 %), Heptacosane (0.22%), Cholest-5-en-3-ol (3.beta.)- (0.62%), Stigmasterol (1.01 %) and gamma.-Sitosterol (3.54 %) were main responsible in effective a ntibacterial activity in methanolic extract (Table 2; Fig. 2).

The current research contributes to the prediction of the formula and composition of 46 biomolecules of methanolic extract of *Z.ovalifolium* with antibacterial activity. Further research may result in the isolation of bio-active compounds, as well as their structural characterization and pharmacological activity testing, which will be useful for future clinical trials.

5. CONCLUSION

Medicinal plants are natural repositories for a variety of phytonutrients and compounds that are essential for life to exist. In the current research, the various solvent extracts of *Z. ovalifolium* fruit demonstrated significant antibacterial activity against *P. aeruginosa, P. syringe, S. aureus, S. typhi, K. pneumonia, and E. coli* in the current research. The various solvent extracts of *Z. ovalifolium* fruit demonstrated significant antibacterial activity against *P. aeruginosa, P. syringe, S. aureus, S. typhi, K. pneumonia, and E. coli* in this research. Among the three solvents used to extract the leaves, methanolic fruit extract demonstrated strong antibacterial activity, compared to hexane and ethyl acetate. G CMS analysis revealed the presence o f antibacterial compounds in the fruit extract of *Z. ovalifolium* such as, 2-cyclopenten-1-one,2-hydroxy (0.67 %), Glycerin (6.08%), 1,2,3-propanetriol, monoacetate (0.64%), Benzofuran,2,3-dihydro (0.21%), E icosanoic acid (0.44%), Octadecanoic acid (Z)-, methyl ester (0.97%), Eicosane (0.40%), E,E,Z-1,3,12-N onadecatriene-5,14-diol(1.65%), Heptacosane (0.22%), Cholest-5-en-3-ol (3.beta.)-(0.62%), S tigmasterol (1.01%) and gamma.-Sitosterol (3.54 %) were mainly responsible in

the antibacterial activity of methanolic extract. The influence of antimicrobial activity of hexane and ethyl acetate will be evaluated further. The methanolic fruit extract must be evaluated for cytotoxic activity and invivo studies f or its effectiveness in suppressing the pathogenic bacteria.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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