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Research Article

GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS OF METHANOL EXTRACT OF *ZANTHOXYLUM RHETSA* DC. FRUITS

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ABSTRACT

Objective: The aim was to investigate important bioactive compounds, biological activities, and medicinal importance of *Zanthoxylum rhetsa* fruits.

Methods: The present work was carried out by gas chromatography–mass spectrometry (GC-MS) method for the identification of different compounds.

Result: The methanolic extract of fruits showed 32 chemical compounds which are identified through GC-MS analysis. Among them, some of the compound names and percentage values are as follows: 2-propanone, 1,3-dihydroxy (48.9%), 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (33.7%), 2-furancarboxaldehyde, 5-[hydroxymethyl] (50.2%), 1-Heptatriacontanol (34.4%), 9,12-octadecadienoic acid (zz)- (48.6%), cholestan-3-ol,2-methylene, [3β,5α] (75.0%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (90.8), 2-furancarboxaldehyde, 5-[hydroxymethyl] (92.0%), hydroquinone (64.9%), n-hexadecanoic acid (37.5%), octadecanoic acid (28.2%), 9,12,15, octadecatrienoic acid, 2-[(trimethylsiyl]oxy]-1-[(trimethylsiyl]oxy]methyl] ethyl ester, [zzz] (22.6%), 9-hexadecanoic acid (10.3%), digitoxin (18.8%), 8,11,14-eicosatrienoic acid methyl ester, [zzz] (25.5%), and oleic acid (16.5%). Most of the identified compounds in the crude methanolic extracts exhibit some bioactivities, namely anticancer, anti-inflammatory, antimicrobial, hepatoprotective, antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-alpha-reductase inhibitor, insectifuge, antiarthritic, anti-coronary, cardiovascular, anti-breast cancer, aromatic, and insectifuge. On the basis of the above investigation, the fruits can be recommended as a treasure of bioactive compounds and it plays a promising role in herbal medicine.

Conclusion: The present study reveals that fruits of *Z. rhetsa* contain various bioactive compounds. Digitoxin is recorded in the ripened fruit of *Z. rhetsa* and it shows the anticancerous and cardiac arrest properties. Hence, in future, this plant will play a promising role in curing cancer.

Keywords: *Zanthoxylum rhetsa* fruits, Gas chromatography–mass spectrometry analysis, Bioactive components.

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INTRODUCTION

Medicinal plants play an important role in human life. Most of the natural products produced by plants are benefiting for humankind, as a food, cosmetics, clothing, building material, tools, and medicines. Most of the medicinal plants are the important source for the discovery of new drugs and drug development. Most of the plant drugs are the secondary metabolites, and it is very much useful for mankind [1]. According to the World Health Organization (WHO), 70–80% of the developing country populations are using traditional plant products as an alternative or complementary medicine [2]. The genus *Zanthoxylum* is belonged to family Rutaceae and economically important because of their alimentary, industrial, and medicinal applications [3,4]. *Zanthoxylum* comprises about 549 species distributed worldwide mainly in tropical and temperate regions [5]. The genus *Zanthoxylum* has enormous importance due to its ethnobotanics, phytochemistry, and biological activity, and it is a promising source of various secondary metabolites. *Zanthoxylum rhetsa* is a medium-sized, deciduous, aromatic, and medicinal tree commonly known as tirphal or chirphal in adjoining area of Kolhapur district. The fruits are aromatic and possess properties such as a stimulant, astringent, stomachache, and digestive. It is also prescribed for urinary diseases, dyspepsia, and diarrhea [6]. This species has potential pharmaceutical applications include cancer treatment, antioxidant, anticoagulant, and antibacterial agents. At the industrial level, species have been shown to contain high amounts of linalool [7], a compound used commercially as a precursor to Vitamin E production, for making soaps, detergents, and insecticides. Screening of the bioactive constituents leads to the development of new novel drugs for curing various maladies [8].

In the present investigation, we have reported important bioactive compounds such as digitoxin, 9 12-octadecadienoic acid (zz)-, 1-heptatriacontanol, n-hexadecanoic acid, and octadecanoic acid. Hence, the present work can act as an activated vein for tribal people for their economic and medicinal purpose. Considering the importance of these compounds in medicine, the present study is necessary.

MATERIALS AND METHODS

Collection of plant material

Plants materials were collected in flowering and fruiting stages in June–February from adjoining areas of Kolhapur district. The specimens were identified by utilizing taxonomic keys and regional floras and authenticated at Angiosperm Taxonomy laboratory, Shivaji University, Kolhapur. The herbariums were prepared by the standardized method and deposited at the Shivaji University Herbarium (SUK) (Collection No.-KDG4).

Preparation of plant extracts

The collected plant materials were transferred immediately to the laboratory and cleaned with water. Selected plant parts were separately shade dried until weight has been constant [9]. The powder for further analysis was prepared using grinder [10], then passed through 40 mm mesh, and stored in a clean container for further use [11]. The extraction was done with the help of methanol using the Soxhlet apparatus [12] for 18 h at a temperature not exceeding the boiling point of the respective solvent [13,14]. The obtained extracts were filtered using Whatman No. 1 filter paper, concentrated using an evaporator at 40°C [15], and stored the residual extracts in the refrigerator at 4°C in small and sterile amber color glass bottles [16].

Gas chromatography–mass spectrometry (GC-MS) analysis

GC-MS analysis was carried out by Hema *et al*. [17] method. Its model name QP 2010 series, Shimadzu, Tokyo, Japan, has well equipped with a VF-5 m fused silica capillary column of 60 m length, 0.25 mm diameter, and 0.25 mm film thickness [18], and it was used during GC-MS analysis. 2 mL of respective diluted samples was manually injected in the splitless mode, with split ratio of 1:40 and with mass scan of 50–600 am. Its linear velocity, pressure and purge flow was 0.289 m/s, 173.3 k Pa and 3.0 mL/min respectively. For GC-MS detection [GC-2010], an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate - total flow: 16.3 mL/min and column flow: 1.21 mL/min. Injector and mass transfer line temperature were set at 200°C and 240°C, respectively. The oven temperature was programmed (column oven temperature: 100°C and injection temperature: 270°C) from 70°C to 220°C at 283.15/min, held isothermal for 60 s, and finally raised to 300°C at 10°C/min. 2 mL of respective diluted samples was manually injected in the splitless mode, with split ratio of 1:40 and with mass scan of 50–600 am. Total running time of GC-MS for mature fruit sample was recorded between 4.39 and 34.36 min, and for ripened fruit, it was from 8.18 to 34.36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas, software adapted to handle mass spectra, and chromatograms was a turbomass. The relative percentage of each extract constituents was expressed as a percentage with peak area normalization. Identification of components and interpretation on mass spectrum of GC-MS were done using the database of the National Institute of Standard and Technology NIST-08 LIB and WILEY-8 LIB. Library. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test material were ascertained [19].

RESULTS

GC-MS of methanol extracts of *Z. rhetsa* mature fruit

In the present investigation, the fruit powder of *Z. rhetsa* was extracted by the methanolic solvent, and it showed pale yellow-colored oil. GC-MS analysis of the methanol extract of the mature fruit of *Z. rhetsa* showed fifteen peaks. The constituents are depicted in Figs. 1-8 and Table 1 with their retention time (RT), molecular formula, molecular weight, area, probability percentage, and important activity. Higher probability percentage was recorded in 2-furancarboxaldehyde, 5-[hydroxymethyl] (50.2%), 2-propanone, 1, 3-dihydroxy (48.9%), 9, 12-octadecadienoic acid (zz) (48.6%), tricyclo [20.8.0.0 [7,16] triacontane, 1 [22], 7 [16] diepoxy (38.3%) and 1-heptatriacontanol (34.4%), respectively, while the remaining of the probability range lies from 33.7% to 8.59%.

Table 1: The compounds identified from the methanol extract of the mature fruit of *Zanthoxylum rhetsa* by gas chromatography-mass spectrometry (GC-MS)

R.T.	Name of compound	Area	Probability	Activity	MF	MW
4.39	2-Propanone, 1,3-dihdroxy	12481	48.9	-	$C_3H_6O_3$	90
4.58	1,3-Diethoxy-2-methylenepropane	10417	18.3	-	$C_8H_{16}O_2$	144
7.02	1H-Azonine, octahydro-1-nitroso	25478	14.9	Antibacterial[20]	$C_8H_{16}N_2O$	156
8.17	4H-Pyran-4-one, 2,3-dihydro -3,5-dihydroxy -6-methyl	8268	33.7	Aroma compound [21]	C_6H8O_4	144
9.70	2-Furancarboxaldehyde, 5-[hydroxymethyl]	29220	50.2	Antimicrobial, preservative [22]	$C_6H_6O_3$	126
13.73	1,3-Propanediol, 2-ethyl-2-[-[hydroxymethyl]	74515	13.3	-	$C_6H_{14}O_3$	134
24.32	Choleston-3-ol, 2-methylene-[3β,5α],	38425	9.78	Antioxidant[23]	$C_{28}H_{48}O$	400
24.60	Oleic acid	32354	22.7	Cardiovascular effects, effects on tumor tissue [24]	$C_{18}H_{34}O_2$	282
25.86	Card-20 (22) enolide, 3,5,14,19-] tetrahydroxy-,[3β,5α]	22824	8.59	-	$C_{23}H_{34}O_6$	406
26.95	Tricyclo[20.8.0.0[7,16]triacontane, 1[22],7[16]-diepoxy	26203	38.3	-	$C_{30}H_{52}O_2$	444
28.27	9,12,15-Octadecatrienoic acid] 2-phenyl-1,3-dioxan-5-yl ester	41004	12.1	-	$C_{28}H_{40}O_4$	440
28.61	1-Heptatriacontanol	45203	34.4	Antioxidant, anticancer, anti-inflammatory, and sex hormone activity [25]	$C_{37}H_{76}O$	536
28.81	9 12-octadecadienoic acid (z z)-	43880	48.6	Anti-inflammatory Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge [26]	$C_{16}H_{32}O_2$	280
30.76	9,12,15, Octadecatrienoic acid 2-[(trimethylsiyl] oxy]-1-[(trimethylsiyl] oxy] methyl] ethyl ester,[ZZZ]	42383	30.8	Antimicrobial [27]	$C_{28}H_{40}O_4$	440
31.10	6β-Hydroxyfluoxymesterone	42849	17.4	-	$C_{20}H_{29}FO_4$	352

M.F: Molecular formula, M.W: Molecular weight. Probability is in percentage (%)

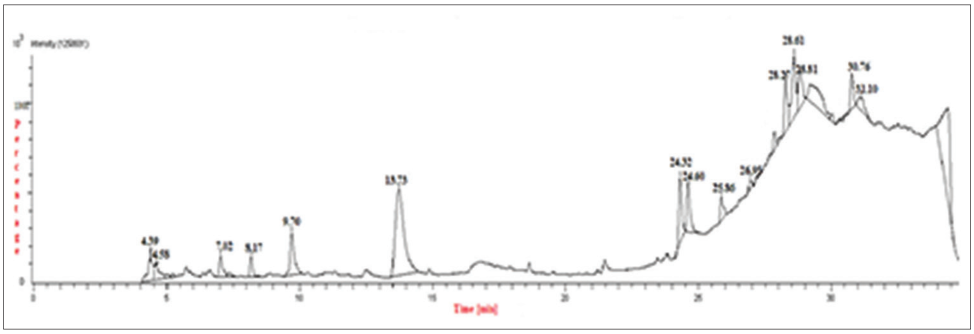

Fig. 1: Gas chromatography–mass spectrometry chromatogram of the methanol extract of *Zanthoxylum rhetsa* mature fruit

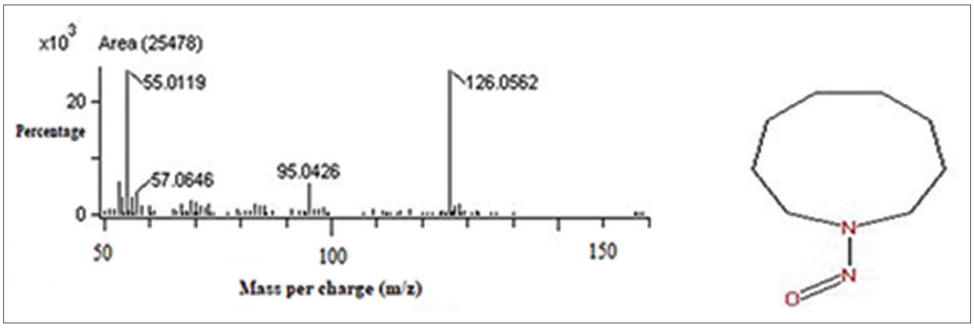

Fig. 2: Best hit and chemical structures of 1H-azonine, octahydro-1-nitroso

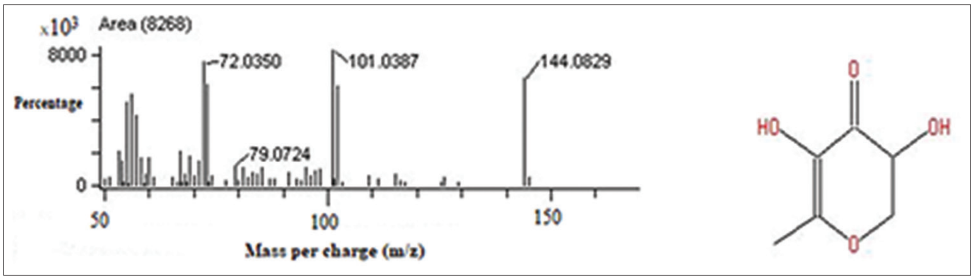

Fig. 3: Best hit and chemical structures of 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy -6-methyl

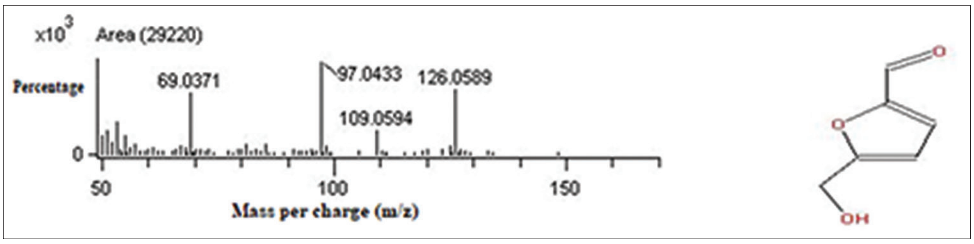

Fig. 4: Best hit and chemical structures of 2-furancarboxaldehyde, 5-[hydroxymethyl]

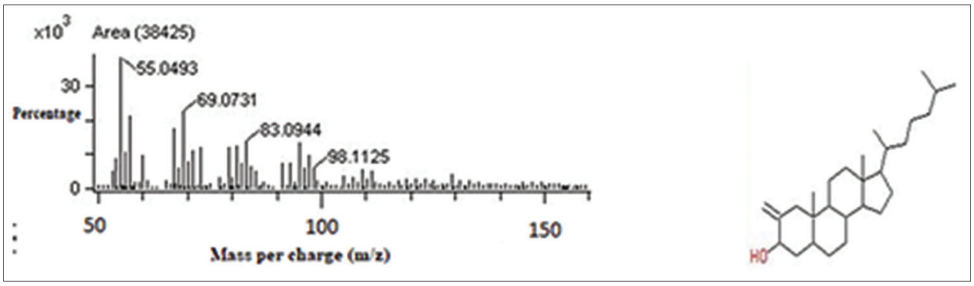

Fig. 5: Best hit and chemical structures of cholestan-3-ol,2-methylene-[3β,5α]

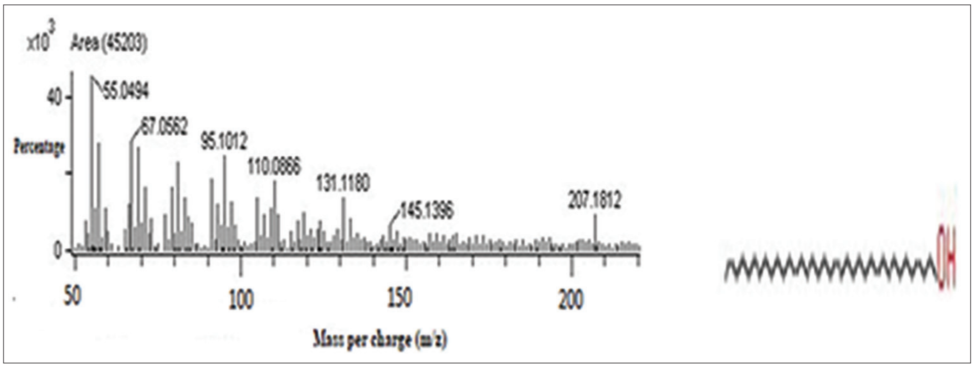

Fig. 6: Best hit and chemical structures of 1-heptatriacontanol

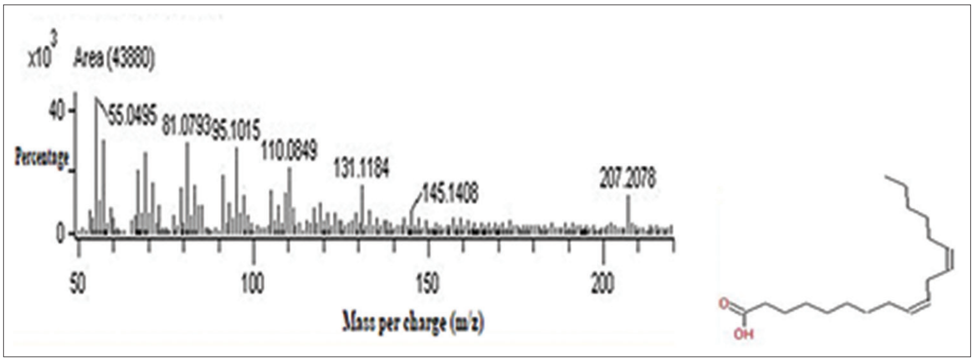

Fig. 7: Best hit and chemical structures of 9 12-octadecadienoic acids (z z)-

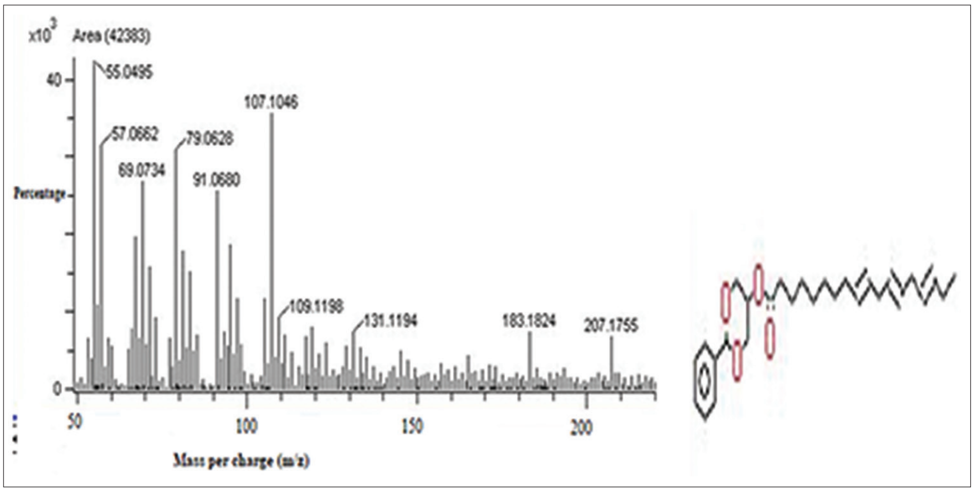

Fig. 8: Best hit and chemical structures of 9, 12, 15, octadecatrienoic acid, 2-[(trimethylsiyl] oxy]-1- [(trimethylsiyl] oxy] methyl] ethyl ester, [ZZZ]

GC-MS of methanol extracts of *Z. rhetsa* ripened fruit

GC-MS chromatogram of the methanol extract of the ripened fruit of *Z. rhetsa* showed fifteen peaks. The constituents are depicted in Figs. 9-16 and Table 2 with their RT, molecular formula, molecular weight, area, probability percentage, and important activity. Higher probability percentage was recorded in 2-furancarboxaldehyde, 5-[hydroxymethyl] (90.2%), H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy -6-methyl (90.8), hydroquinone (64.9%), n-hexadecanoic acid (37.5%), and octadecanoic acid (28.2%); remaining ones are lies in the range between 10.3% and 25.5%.

In the present investigation, five compounds, namely 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, 2-furancarboxaldehyde, 5-[hydroxymethyl], oleic acid, tricyclo [20.8.0.0[7,16] triacontane,1[2],7[16]-diepoxy 9,12,15, octadecatrienoic acid, and 2-[(trimethylsiyl]oxy]-1-[(trimethylsiyl]oxy] methyl] ethyl ester [ZZZ], were observed in mature as well as the ripened fruit of *Z. rhetsa.*

DISCUSSION

GC-MS analysis and biological evaluation of essential oil of *z. rhetsa* were performed [36]. In this investigation, they have focused on the essential oil composition of the pericarp of *Z. rhetsa.* They have reported one of the important essential oils, i.e. hexadecanoic acid, and it is well known for its antimicrobial activity. In the present study, n-hexadecanoic acid and 9-hexadecanoic acid were recorded.

Characterization of the essential oil and fatty oil from the fruit of *Z., rhetsa* was carried out [37]. They have mentioned important fatty oils such as (Z)-9-hexadecenoic acid methyl ester, hexadecanoic acid methyl ester, (Z)-

Table 2: The compounds identified from the methanol extract of the ripened fruit of *Zanthoxylum rhetsa* by GC-MS

R.T.	Name of compound	Area	Probability	Activity	MF	MW
7.01	Cyclohexnamine, N-3-butenyl- N-methyl	2527	16.5	-	$C_{11}H_{21}N$	167
8.17	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy -6-methyl	18341	90.8	Antimicrobial, Anti-inflammatory [28]	$C_8H_8O_4$	144
9.70	2-furancarboxaldehyde, 5-[hydroxymethyl]	89250	92.0	Antimicrobial, preservative [28]	$C_6H_6O_3$	126
10.99	Hydroquinone	30824	64.9	Depigmenting agent [29]	$C_6H_6O_2$	110
13.65	Sucrose	38057	18.5	-	$C_{12}H_{22}O_{11}$	342
21.48	n-hexadecanoic acid	14801	37.5	Antioxidant, [30]	$C_{16}H_{32}O_2$	256
24.32	trans-13-Octadecenoic acid	59668	10.5	-	$C_{18}H_{34}O_2$	282
24.62	Octadecanoic acid	65759	28.2	Drug delivery system [31]	$C_2H_{36}O_2$	284
25.86	9,12,15, Octadecatrienoic acid 2-[(trimethylsiyl] oxy]-1-(trimethylsiyl], oxy] methyl] ethyl ester,[ZZZ]	20685	22.6	Antimicrobial [27]	$C_{27}H_{52}O_4S_{i2}$	496
28.28	10,12,14,-nonacosatriynoic acid	36372	11.6	-	$C_{29}H_{46}O_2$	426
28.61	7,10,13,-Eicosatrienoic acid	45460	17.5	Primary metabolites of acetyl CoA pathway[32]	$C_21H_{36}O_2$	320
28.79	9-hexadecanoic acid	46400	10.3	Antimicrobial [27]	$C_{16}H_{30}O_2$	254
29.57	Benz[e] azulene-3,8-dione, 5[(acetyloxy) methyl]-	37353	16.4	-	$C_{19}H_{24}O6$	348
30.13	Digitoxin	40385	18.8	Anticancer agent and cardiac glycoside [33]	$C_{41}H_{64}O_{13}$	764
30.76	8,11,14-eicosatrienoic acid, methyl ester,[zzz]	40629	25.5	Aromatic compound[34]	$C_{21}H_{36}O_2$	320
31.57	Oleic acid	36972	16.5	Protecting breast cancer [35]	$C_{18}H_{34}O_2$	282
32.51	Tricyclo[20.8.0.0[7,16]triacontane, 1[2],7[16]-diepoxy	41335	11.2	-	$C_{30}H_{52}O_2$	444

M.F: Molecular formula, M.W: Molecular weight. Probability is in percentage (%)

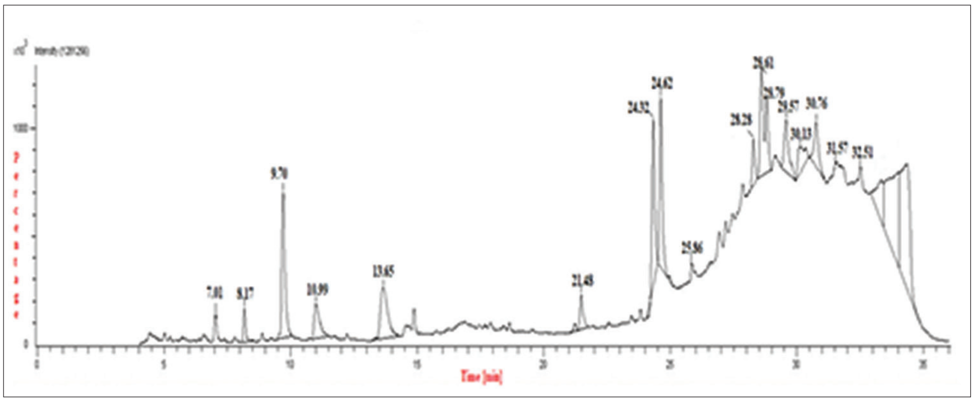

Fig. 9: Gas chromatography–mass spectrometry chromatogram of the methanol extract of *Zanthoxylum rhetsa* ripened fruit

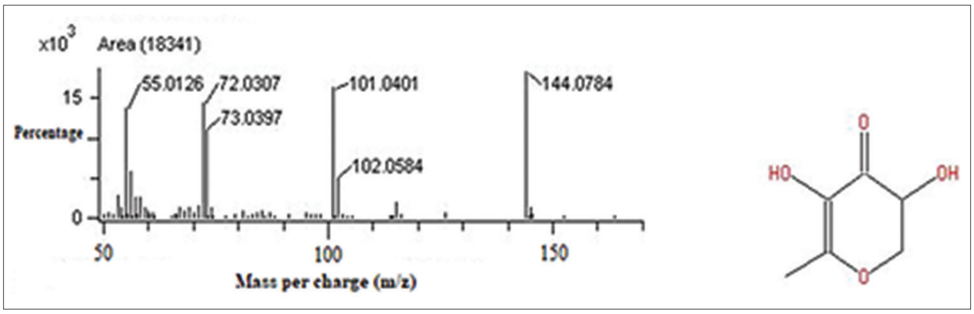

Fig. 10: Best hit and chemical structures of 4H-pyran-4-one, 2, 3-dihydro 5[hydroxymethyl]

11-octadecenoic acid methyl ester, (Z,Z), 9,12-octadecadienoic acid methyl ester,(ZZZ), 9,12,15-octadecatrienoic acid methyl ester, 11-eicosenoic acid methyl ester, and essential oil such as thujene, α-pinene, sabinene, β -myrcene, β- pinene, 3-career, p-cymene, limonene, ocimene, and γ- terpinene. In the present investigation, 9,12,15, octadecatrienoic acid, 2-[(trimethylsiyl] oxy]-1-[(trimethylsiyl]oxy] methyl] ethyl ester[ZZZ], 8,11,14-eicosatrienoic acid methyl ester,[zzz], 7,10,13,-Eicosatrienoic acid methyl ester, 9 12-octadecadienoic acid (z z)-, 9,12,15-Octadecatrienoic acid, and 2-phenyl-1,3-dioxane-5-yl ester were reported.

Digitalis therapy in patients with congestive heart failure was carried out [38]. According to them, digitalis contains four important

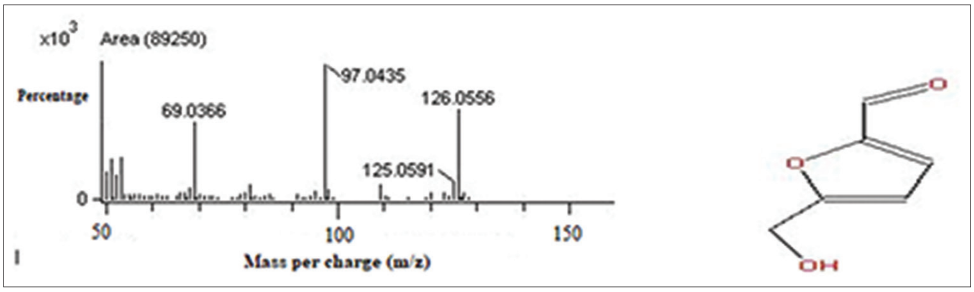

Fig. 11: Best hit and chemical structures of 2-furancarboxaldehyde, 5[hydroxymethyl]

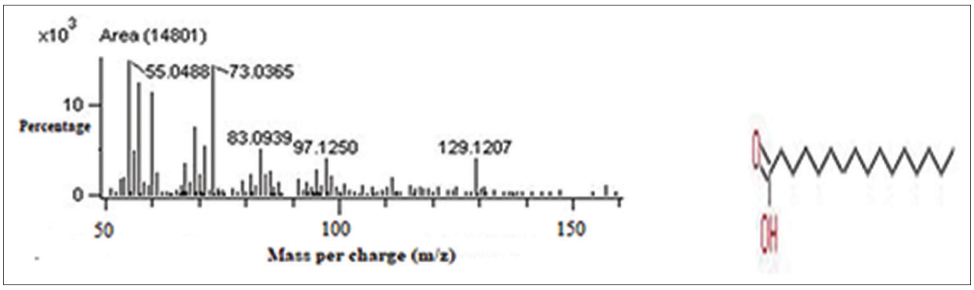

Fig. 12: Best hit and chemical structures of n-hexadecanoic acid

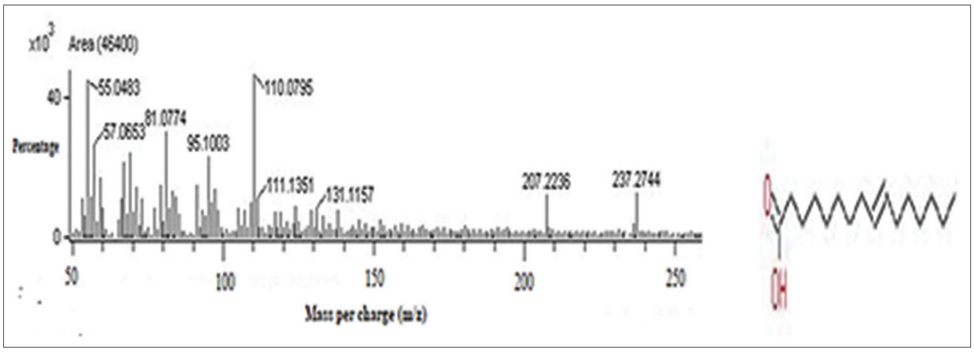

Fig. 13: Best hit and chemical structures of 9-hexadecanoic acid

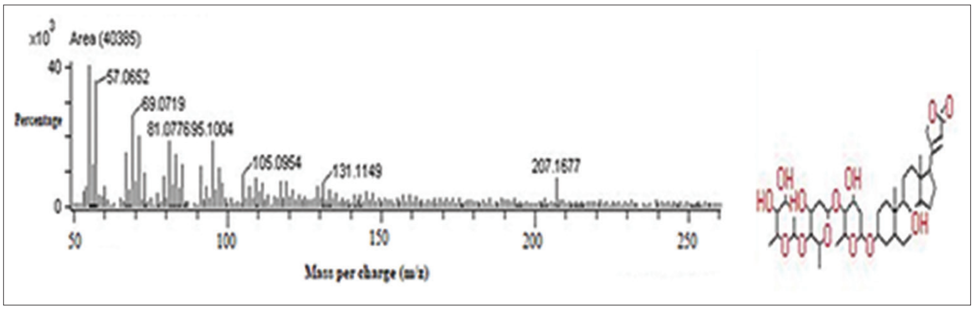

Fig. 14: Best hit and chemical structures of digitoxin

glucosides such as digitalin, digitale, digitonin, and digitoxin. The digitoxin is most powerful, extremely poisonous, and cumulative drug. The present study showed the presence of digitoxin (18.8%) in the ripened fruit of *Z. rhetsa*.

Bioactive constituents of *Z. rhetsa* bark and its cytotoxic potential against B16-F10 melanoma cancer and normal human dermal fibroblast (HDF) cell lines were carried out [39]. They have mentioned the isolation of major volatile constituents such as tetrahydrofuran lignans (yangambin and kobusin), berberine alkaloid (columbamine), and a triterpenoid (lupeol) from the bark of *Z. rhetsa.* They tested the solvent fractions and purified compounds for their cytotoxic potential against HDF and mouse melanoma (B16-F10) cells, using the MTT assay. The chloroform fraction and kobusin exhibited a cytotoxic effect against B16-F10 melanoma cells. In the present study, digitoxin is recorded in the ripened fruit of *Z. rhetsa,* and it shows the anticancerous and cardiac arrest properties. Hence, in future, this plant will play a promising role in curing cancer.

GC-MS analysis of the hexane extract of *Zanthoxylum armatum* fruits was carried out [40]. They have recorded 36 chemical compounds. Some of them are as follows Eucalyptol, linalool, quercitron, menthoglycol, trans-pipertiol, carveole, exo-2-hydroxy-1 and 8-cineole, the aspirane,

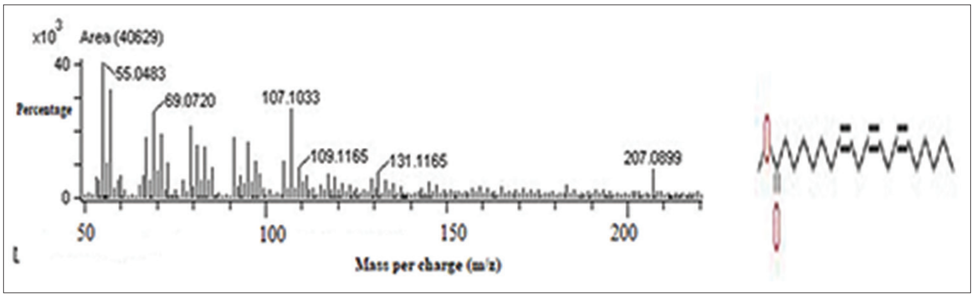

Fig. 15: Best hit and chemical structure of oleic acid

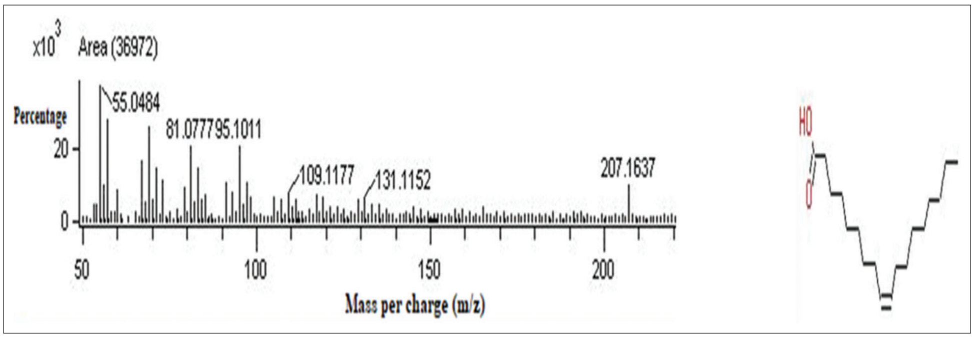

Fig. 16: Best hit and chemical structure of tricyclo [20.8.0.0[7, 16] triacontane, 1[2], 7[16]-diepoxy

piperitone, cuminol, artemisia alcohol, caryophyllene, α-asarone, β-asarone, 2-hydroxy cyclopentadecanone, palmitic acid, and palmitic acid methyl ester. In the present study, 32 chemical compounds were recorded in the mature and ripened fruit of *Z. rhetsa.* They have not mentioned the stage of fruit.

Estimation of some secondary metabolites from the *in vitro* cultures of C*hlorophytum borivilianum* was carried out [41]. They have mentioned 23 different types of biologically active compounds. Among these compounds, digitoxin is one of them. In the present study, digitoxin (18.8%) is reported in the ripened fruit of *Z. rhetsa*.

CONCLUSION

In the present study, 32 compounds were present in the methanolic extract of *Z. rhetsa* fruit; among them, 15 compounds recorded in mature fruit, whereas 17 compounds in the ripened fruit. The recorded compound showed various biological activities such as anticancerous, antimicrobial, antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic, anti-inflammatory and anti-cardiac arrest activities. It also provides a potential source of the industrial application. One of the commercially important bioactive compounds digitoxin present in the ripened fruit which is mostly used for heart diseases. On the basis of the above results here with concluded that fruit of *Z. rhetsa* medicinally important.

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AUTHORS' CONTRIBUTION

The author carried out the experiment and wrote the manuscript with support from Dr.V. D. Jadhav. Dr. V. D. Jadhav also supervised the manuscript.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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