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GC-MS Analysis and Biological Evaluation of Essential Oil of Zanthoxylum Rhesta (Roxb.) DC Pericarp

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ABSTRACT

The present study reports the chemical composition, antioxidant, antibacterial, antidiarrheal as well as the spasmolytic activity of *Zanthoxylum rhetsa* (DC) essential oil, fractionated oil and its principal constituent (Terpinen-4-ol). The constituents of essential oil were characterized by GC-FID and GC-MS. The pharmacological and biological activities of oil, its fraction and principal constituent were carried out in-vivo and *in-vitro*. The oil was rich in a group of monoterpene family, constituted mainly of terpinen-4-ol (25.43%), sabinene (16.50%), β-pinene (10.4%), α-Terpineol (7.63%), γ-Terpinene (5.64%), α-pinene (4.33%), and linalool (3.25%). The antioxidant capacities of the oil, fractions and terpinen-4-ol were assessed by using spectrophotometry to measure free radical scavenger 2,2-diphenyl-1-picrylhydrazyl (DPPH). Furthermore, the oil, its fractions and terpinen-4-ol exhibited appreciable antioxidant, antibacterial, antidiarrheal and non-selective spasmolytic activity. The study suggests that the oil and its main active constituent (terpinen-4-ol) of the studied plant would have high potential in the treatment of stress and gastrointestinal diseases.

Keywords: *Zanthoxylum rhetsa*, GC-MS, Antioxidant, DPPH, Antibacterial, Antidiarrheal, Spamolytic activity.

1. INTRODUCTION

The demand for natural product and formulation is increasing due to the high risk of side effects posed by the synthetic drugs. Studies suggest that the synthetic antioxidant like butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and *tert.*-butyl hydroxyquinone (TBHQ) have potential to promote cancer in laboratory animals like rat[1]. The prolonged use of synthetic antioxidants is restricted due to their side effect. This has prompted researchers all over the world to look for a natural and safe antioxidant. The compounds from plant products are safer as they produce less toxic or inactive metabolites. It is reported that various ailments (such as atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer), are treated with natural drugs and antioxidant based formulation. Spices and herbs contain various antioxidants, and chemical constituents which are used for treating various diseases and aging [2].

Zanthoxylum rhetsa (Roxb.) DC (Hindi Name Trifal), is a small deciduous tree that belongs to the family Rutaceae, commonly grown wildly in coastal Karnataka, southern part of Maharashtra and other parts of India. Tree bears green color fruits which turns dark brown to black upon drying, exposing the seed which is discarded. The grinded pericarp is used as condiment in fish and some vegetables curries to enhance the flavor in Karnataka State (India). The pericarp contains essential oil, which has pleasant odor similar to sweet orange,

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while unripe pericarp has aromatic taste like orange rind. It gives pungent tingling taste due to hydroxyl-α-sanshool, present in the oil[3-5]. Sabinene (35.7-67.7%) is the major constituent in the essential oil, which is responsible for anti–inflammatory, anesthetic and hypotensive activities[6]. It has been reported that essential oil has comparatively better anthelmintic activity against *Taenia solium, Ascaridia galli* and *Pheretima posthuma* when compared with synthetic compound like piperazine phosphate[7]. Local community used the essential oil in the treatment of cholera[8]. Essential oil is used as antiseptic, disinfectant and for the treatment of asthma, toothache and rheumatism[9]. The major constituents present in the essential oil of seed coat were terpinen-4-ol (32.1%), α-terpineol (8.2%), sabinene (8.1%), β-phellandrene (7.4%) and 2-undecanone (7.1%)[10]. Sabinene (66.3%), α-pinene (6.6%), β-pinene (6.3%) and terpinen-4-ol (3.5%) were the major components of the seed oil[5]. The pH of extraction medium was noted to influence the essential oil composition of *Z. rhetsa* seeds[11]. A variety of reports are available in literature on the composition and the biological activity of the plant extracts and essential oil. Literature survey reports the antidiarrheal activity of extract of stem bark only[12], however, to the best of our knowledge the antidiarrheal and spasmolytic activities of essential oil has not been studied so far. The secondary metabolites vary according to the geographical and environmental conditions; in the present study, we report the chemical constituents of essential oil of pericarp and the antioxidant, antibacterial, antidiarrheal and spasmolytic activity associated with the oil and active compound isolated from the *Z. rhetsa* wildly grown in the southern-western coast of India.

Experimental

Plant materials (Trifal seeds with pericarp) were collected from trees grown in Karwar City, Karnataka, India (Western Coast) during August-October 2012 with the help of local people and was authenticated by M.P. Srivastav, Retired Botanist, Sagar University, Sagar (India). Herbarium specimen (H-2013-1) is deposited at Faculty of Pharmacy, Al-Ahliyya Amman University, Jordan. Plant material consisting of mature fruits was dried at room temperature. After separation from the seeds, pericarp were grounded using a mixer. Ascorbic acid, α-pinene, β-pinene, sabinene, γ-terpinene, linalool, terpinen-4-ol, α-terpineol and DPPH were purchased from Sigma-Aldrich, USA. Analytical grades solvents were used for the experiments.

Experimental animals including Albino mice (20–22 g) and New Zealand guinea-pigs (300–450 g) were purchased from local market. All animals were acclimatized (temperature 25±2 °C; humidity 60%) for 10 days, had free access to water and food. All experiments on animals were conducted as per the ethical guidelines.

Isolation of essential oil and preparation of stock solution

One hundred gram of grounded pericarp powder was subjected to hydro-distillation with 1 L of water using a Clevenger-type apparatus (JSOW, India) for 4 hours. The oil obtained for each specimen were dried with anhydrous sodium sulphate, pooled and stored between 4-8°C in amber glass vials until analysis. The yield of the oil was found to be 2.01 ml/100 g of plant material. About 20 ml of essential oil was collected by using hydro-distillation. Stock solutions of essential oil were prepared by dissolving a known amount of oil in 98% methanol. The working solutions were prepared (0.98 to 1000 µg/mL) for antioxidant activities using suitable dilution in methanol. For other activities samples were prepared in 30% aqueous solution of dimethylsulfoxide.

Analysis of Essential Oil

Analysis of essential oil and fractions were carried out using a Hewlett Packard (5890 Series II gas chromatograph equipped with DB-5MS fused silica column (5% Phenyl, 95% polydimethylsiloxane 30m x 0.25 mm, film thickness 0.25 µm), interfaced with a Hewlett Packard mass selective detector 5972, operated by HP Enhanced Chemical Software, version A.03.00. Oven temperature was programmed: 60 °C (0-5 min.); 60-240 °C (4 ºC/min.); 240 °C (10 min.), Injector

temperature: 280 °C; carrier gas: helium; injection volume (1 µL, splitting ratio 1:50, sample diluted with dichloromethane (1:10)); MS source temperature/Detector temperature: 280 °C, ionization energy: 70 eV; amu gain-492, amu offs.-67, ionization current 60µm; scan range 50-550 amu. Mass spectrum of every chemical constituent was compared with the corresponding reported spectrum in NIST, Wiley Mass Spectral Database (1995) for GC-MS and published references.

Identification of compound was confirmed by comparing its retention indices (RI) relative to *n*-alkanes (C_{10}-C_{40}) and reference data[13-14]. Co-Chromatographic analyses (CO-I)[15-16] of oil with the authentic samples (α-pinene, β-pinene, sabinene, γ-terpinene, linalool, terpinen-4-ol, α-terpineol and hexadecanoic acid) further supported the identifications. GC analysis of essential oil and fractionated oil samples were conducted using a Shimadzu-GC-2010 gas chromatograph equipped with FID using similar chromatographic conditions. The injector and detector temperature were kept at 220 ºC and 300 ºC respectively. Relative percentages of the eluted components are reported without the use of correction factor.

Fractionation of Essential Oil

Essential oil of *Z. rhetsa* (10 ml) was fractionated using normal phase column chromatography using silica gel (Davisil grade, Sigma Aldrich, USA) and gradient elution with a mixture of solvents (n-hexane, n-hexane-ethyl acetate). Fractions were collected and concentrated at 35-40ºC, and analyzed by thin layer chromatography. Similar fractions according to TLC profiles were pooled to yield 25 fractions. Fractions (2–5 and 14–16) which possessed antibacterial activity were also evaluated for other activities. Fraction (14-16) was further purified by preparative thin layer chromatography (2 mm thickness) or column chromatography to yield a pure compound (**1**). The structure of compound (**1**) was elucidated using ^{1}H-NMR and GC-MS and identified as terpinen-4-ol. Colorless oil, ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 0.90-98 (6H, d, J=6.1 Hz, 2xCH_3), 1.55-1.65 (3H, m, CH_3-C*H*-CH_3, and -C-C*H*$_2$-CH_2-), 1.75 (3H, br, CH_3); 1.90 (2H, dd, J = 15.0 Hz, 6.0 Hz, -CH_2-C*H*$_2$-C(CH_3)=), 2.11 (br, 1H, OH, D_2O exchangeable), 2.25 (2H, dd, J = 15.0 Hz, 6.0 Hz, -C*H*$_2$-CH=C<); 5.28 (1H, dd, J = 6.0 Hz, 3.0 Hz, -CH_2-C*H*=C<). Experimental data matches with the reported data[17].

Determination of Total Phenolic Content

Total phenolic content was determined using method described by Gutfinger with slight modification[18]. A methanolic solution of sample (100 µg/mL) was prepared. Folin-Ciocalteau's reagent (1/10 dilution, 1mL) was added to sample solution (1 mL). Sodium carbonate (1 mL, 25% w/v) was added to content and diluted to 10 ml using distilled water. The absorbance of resultant solution was measured at 725 nm using Thermo spectrophotometer after incubation at 25 ºC for 1 h. Gallic acid served as a standard for preparation of calibration curve and total phenolic content of the sample is expressed as mg gallic acid equivalent (GAE/g).

Antioxidant activity (DPPH free radical scavenging activity)

The antioxidant activity of the essential oil, fractions, isolated compound and the standard was assessed on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical activity by modified method[2,19]. Ascorbic acid (1-100 µg/mL) was used as standard. Different concentration of samples (3-750 µg/mL) were prepared and evaluated for the activity.

Antibacterial Activity

The antibacterial activity of the oil, fraction and isolated compound was evaluated by agar diffusion method against three bacterial species two gram negative strains *Klebsiella pneumonia* and *Escherichia coli* ATCC 8739 and one gram positive strain, *Staphylococcus aureus* ATCC 6538a. Wells of 7 mm diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50 µl of sample in 30% DMSO (dimethyl sulphoxide) were added in each well. The plates were incubated at 37.0±0.5°C for 24-48 h. Plates were

removed from the incubator and the diameter of the inhibition zone was measured in mm.

In a separate experiment (broth microdilution), the IC_{50} values were determined according to the National Committee for Clinical Laboratory Standards (NCCLS) with some modification. MIC tests were performed in 96 flat bottom microtiter plates (TPP, Switzerland) as reported earlier[20]. Different concentration of samples (3-750 µg/mL) were prepared and evaluated for the activity.

Table 1. Chemical composition of *Z. rhetsa* (Roxb) DC pericarp essential oil.

S. N.	RRI	Identification method*	Name of the compound	Area %
1	923	MS, RI	α–Thujene[a,c]	0.74
2	934	MS, RI, CO-I	α-Pinene[a,c]	4.33
3	976	MS, RI, CO-I	Sabinene[a,c]	16.50
4	980	MS, RI, CO-I	β-pinene[a,c]	10.40
5	991	MS, RI	β-Myrcene[a,c]	0.68
6	1001	MS, RI	Δ^4-Carene[a,c]	0.85
7	1005	MS, RI	1- Phellandrene[a,c]	3.01
8	1026	MS, RI	p-Cymene[a,c]	2.45
9	1031	MS, RI	β-Phellandrene[a,c]	4.37
10	1050	MS, RI	Trans-ocimene[a,c]	0.07
11	1062	MS, RI, CO-I	γ-Terpinene[a,c]	5.64
12	1063	MS, RI	Trans-Sabinene hydrate[a,c]	0.34
13	1070	MS, RI	1-Octanol[c,d]	0.20
14	1088	MS, RI	α-Terpinolene[a,c]	1.48
15	1098	MS, RI, CO-I	Linalool[a,d]	3.25
16	-	-	Unidentified	1.17
17	1101	MS, RI	Cis-p-2-methen-1-ol[a,d]	0.42
18	1163	MS, RI	E-β-terpineol[a,d]	0.64
19	1177	MS, RI, CO-I	Terpinen-4-ol[a,d]	25.43
20	1189	MS, RI, CO-I	α-Terpineol[a,d]	7.63
21	1204	MS, RI	Decanal[c,d]	0.36
22	-	MS (t)	2-Propylcyclopentanone[d,e]	0.81
23	-	MS (t)	Z/E-3,7,-Dimethyl-6-oxo-2-Octenal[a,d]	0.15
24	1220	MS, RI	α-Fenchyl acetate[a,d]	0.07
25	1228	MS, RI	Nerol[a,d]	0.10
26	1239	MS, RI	Cuminal[a,d]	0.06
27	1274	MS, RI	1-Decanol[c,d,e]	0.20
28	1280	MS, RI	Nonanoic acid[e,d]	0.07
29	1282	MS, RI	Trans-piperitone[a,d]	0.24
30	1287	MS, RI	p-Cymen-7-ol[a,d]	0.17
31	1298	MS, RI	Carvacrol[a,d]	0.13
32	-	MS	(E,E)-2,4-Hexadienoic acid[e,d]	0.07

33	-	-	Unidentified	0.09
34	1351	MS, RI	α-Cubebene[b,c]	0.05
35	-	MS	2,3,3-trimethyl-1,4-pentadiene[c,e]	0.20
36	1375	MS, RI	β-Elemene[b,c]	0.07
37	1376	MS, RI	α-Copaene[b,c]	1.09
38	1402	MS, RI	Dodecanal[c,d]	0.05
39	1418	MS, RI	trans-Caryophyllene[b,c]	0.07
40	-	MS	Epi-bicyclosesquiphellandrene[b,c]	0.10
41	-	MS	2-isopropyl-5-methyl-9-methylene-Bicyclo[4.4.0]dec-1-ene[b,c]	0.12
42	1455	MS, RI	α-Humulene[b,c]	0.37
43	1477	MS, RI	γ-Muurolene[b,c]	0.09
44	1499	MS, RI	(-)-α-Muurolene[b,c]	0.07
45	1522	MS, RI	α-Bisabolene[b,c]	0.90
46	1532	MS, RI	(E)-Nerolidol[b,d]	0.07
47	1567	MS, RI	Dodecanoic acid[e,d]	0.11
48	-	-	Unidentified	0.29
49	-	-	Unidentified	0.13
50	1636	MS, RI	τ-Cadinol[b,d]	0.07
51	1654	MS, RI	α-Cadinol[b,d]	0.59
52	-	MS (t)	(-)-4-oxo-14-norvitrane[e]	0.35
53	1711	MS, RI	Farnesol[b,d]	0.13
54	-	MS (t)	Cis-Undec-4-enal[c,d]	0.13
55	1765	MS, RI	Tetradecanoic acid[e,d]	0.11
56	1960	MS, RI, CO-I	Hexadecanoic acid[e,d]	1.14
57	-	MS (t)	Heptadecene-8-carbonic acid[e,d]	0.28
58	1995	MS, RI	Heptadecane[c,e]	0.22

* Compounds were identified using MS and/or Retention Indices (RI). Relative retention Indices (RRI) Lit. [24-25]. CO-I : co-chromatography with authentic sample; - : Unidentified

[a] monoterpene, [b]sesquiterpene, [c] hydrocarbon, [d] oxygenated, [e] nonterpene

Antidiarrheal Activity

Antidiarrheal activity of the essential oil, selected fractions and terpinen-4-ol was investigated using castor oil induced diarrhea method described by Borrelli[21]. The experimental animals (20-25g) were administered: vehicle (1%, carboxymethyl cellulose, control); essential oil (100 µL/kg p.o., suitably diluted with DMSO); isolated Fractions (F3-4, F14-16) and isolated compound (terpinen-4-ol) and standard loperamide (10 mg/kg, p.o., positive control). One hour after the treatment, each animal received castor oil (0.2 mL) through feeding cannula. Two hours after dosing the castor oil, the animals were inspected for (by an observer unaware of the particular treatment) for the presence of unformed water fecal pellets; their absence was recorded as a positive result, indicating protection from diarrhea during the observed period of the experiment.

In-vitro Smooth Muscle Relaxant Activity

Guinea-pigs were killed by asphyxiation with CO_2

and segments (2–3 cm) of the terminal ileum were removed, flushed of luminal contents. Ileum was suspended in an organ bath (capacity 16 mL) containing aerated (95% O_2; 5% CO_2) Tyrode solution (pH 7.4) maintained at 34.0 ± 0.5ºC. Contractions were recorded using isotonic transducer (load 0.5g) connected to Harvard data acquisition system[22]. Spasmolytic activity of the essential oil, selected fractions and terpinen-4-ol (isolated compound) was assessed by their ability to prevent the contraction induced by the submaximal concentration of acetylcholine (2.5×10^{-7}), histamine (3.0×10^{-7}) or nicotine (2.5×10^{-6}, g/mL). In all the isolated preparation, different concentrations of essential oil, fraction and terpinen-4-ol were tested against the spasmogen and the IC_{50} was calculated using Sigma Plot ver 11.0.

Table 2. Composition of the fraction obtained from *Z. rhetsa* pericarp essential oil

	Fractions (Relative %)						
Active constituent ▼	F2	F3	F4	F5	F14	F15	F16
A-Thujene	80.1	1.2	-	-	-	-	-
α-Pinene	8.8	70.5	11.3	9	-	-	-
Sabinene	3.2	15.2	45.5	35.5	-	-	-
β-Pinene	-	2.3	30.4	36.9	-	-	-
β-Myrecene	-	-	-	1.9	-	-	-
γ-Terpinene	-	-	-	-	3.3	-	-
Linalool	-	-	-	-	3.4	-	-
E-β-terpineol	-	-	-	-	5.4	3.2	3.1
Terpinen-4-ol	-	-	-	-	70.5	60.4	10.8
α-Terpineol	-	-	-	-	12.5	20.1	12.4
Other	-	-	-	-	-	16.0	70.8

Table 3. Antioxidant and antibacterial activity of essential oil, fractions and terpinen-4-ol (isolated compound)

Test compounds	***Anti-oxidant activity*$**	***Antibacterial activity# (MIC*** μg/mL,)		
	IC_{50} (μg/mL)* (Mean ± SD)	***S. aureus ATCC 6538a***	***E. coli ATCC 8739***	***K. pneumoniae***
Essential oil (pericarp)	7.5 ± 0.6	35	140	70
F-2	84.0 ± 4.0	-	-	-
F-3	71.5 ± 5.5	140	-	-
f-4	58.9 ± 2.9	70	-	-
f-5	64.2 ± 2.5	140	-	-
f-14	51.3 ± 3.5	-	-	-
f-15	71.4 ± 3.1	70	140	-
f-16	71.4 ± 3.5	140	140	-
Terpinen-4-ol	47.9 ± 2.0	35	70	140
Ascorbic acid	5.1 ± 0.5	-	-	-
Ciprofloxacin	-	0.73	1.46	1.46

$ n=3, # n=2

Table 4. Antidiarrheal activity of essential oil, fractions and terpinen-4-ol (isolated compound)

Treatment (p.o.)	Dose (µL/kg, mg/kg, b.w.)	No. of mice with diarrhea	% protection (p value)
Control (1% CMC+ Castor oil)	-	6/6	0.0
Essential oil	100	2/6	66.7, ($p<0.014$)
Essential oil	200	1/6	83.3, ($p<0.003$)
F-3	100	3/6	50.0, ($p<0.046$)
f-4	100	4/6	33.3, ($p>0.05$)
f-14	100	3/6	66.7, ($p<0.014$)
f-15	100	2/6	66.7, ($p<0.014$)
f-16	100	2/6	50.0, ($p<0.046$)
Terpinen-4-ol	100	2/6	66.7, ($p<0.014$)
Loperamide	100	0/6	100.0, ($p<0.001$)
χ^2 test =difference between control and treated group			

Table 5. Spasmolytic activity of *Z. rhetsa* (Roxb) DC pericarp essential oil, fractions and terpinen-4-ol

Test material/compound	IC_{50} (µg/mL) against spasmogen (Mean ± SD)	
	Acetylcholine (2.5×10^{-7} g/mL),	Histamine (3.0×10^{-7} g/mL)
Essential oil	23.0 ± 1.2	39.0±1.8
F-2	-	-
F-3	70.0 ± 3.1	-
f-4	68.4 ± 5.8	-
f-5	70.0 ± 4.3	-
f-14	60.0 ± 2.5	-
f-15	56.0 ± 5.5	-
f-16	-	-
Terpinen-4-ol	40.0 ± 3.5	-
Atropine	0.02 ± 0.005	-
Cyproheptadine	-	0.05±0.002

Waste Disposal

All the waste material and chemicals were collected in organic waste container and disposed off as per university guideline.

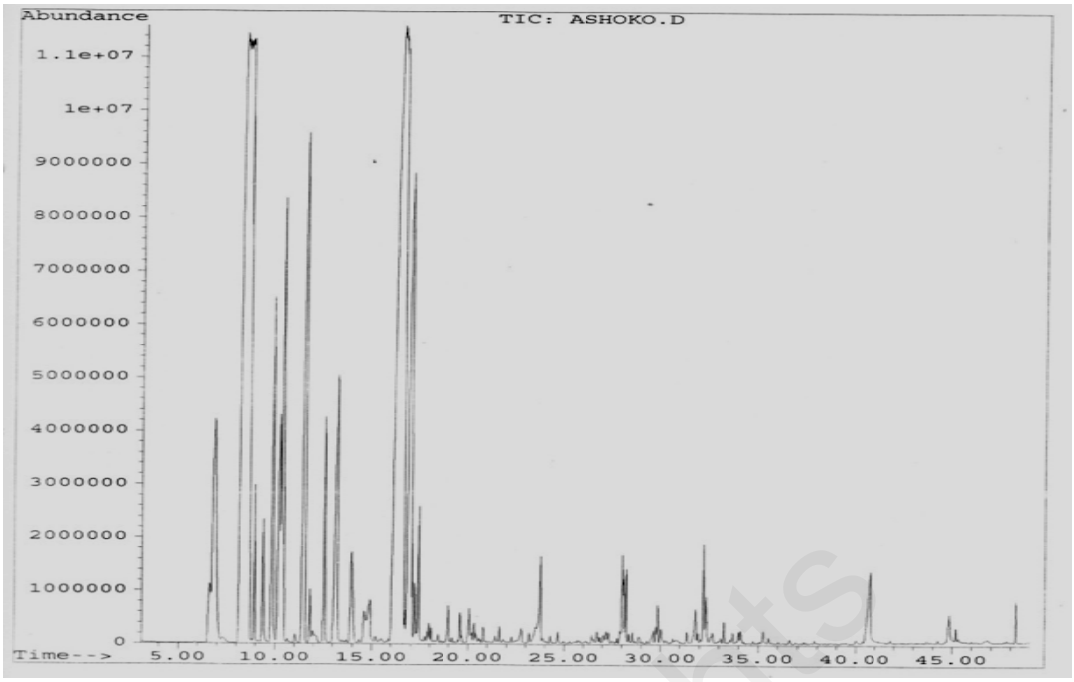

Figure 1. GC-MS chromatogram of essential oil (pericarp) of *Z. rhetsa* (Roxb) DC

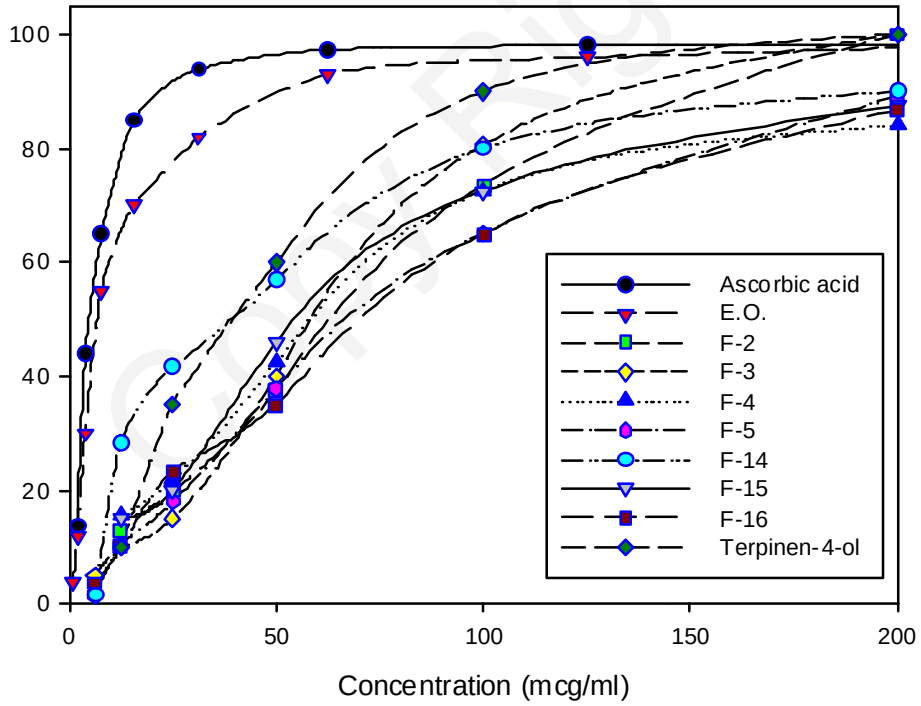

Figure 2. Free radical scavenging activity of essential oil (pericarp), fractions, terpinen-4-ol (isolated compound) and ascorbic acid

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oil

As per the GC and gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil from pericarp of *Z. rhetsa*, the results showed the presence of 58 compounds in the oil, compared 27 components identified in the plant oil by Sawant et al.[23] using HS-GCMS. Fifty three compounds were identified by comparing retention indices, mass spectroscopy and/or by using co-chromatography[13-16]. The retention indices, relative percentage composition are given in Table 1, where the components are listed according to their retention indices on the column. The essential oil contains several different compounds belonging to categories like monoterpene hydrocarbon (~50.5%), oxygenated monoterpene (38.3%), sesquiterpene (~3%), oxygenated sesquiterpene (~0.9%), and other. The oil was rich in a group of monoterpene family, mainly monoterpene alcohol (oxygenated monoterpene), including terpinen-4-ol (25.43%), which was the principal component, α-terpineol (7.63%) and linalool (3.25%); monoterpenes hydrocarbon like sabinene (16.50%), β-pinene (10.4%), γ-Terpinene (5.64%) and α-pinene (4.33%). Other major constituents were β-phellandrene (4.37%), p-cymene (2.45%), α-terpinolene (1.48%) and δ-carene (0.85%). As mentioned earlier the sesquiterpens were less than monoterpens. The main sesquiterpenes were α-copaene (1.09%), α-bisabolene (0.90%) and α-Humulene (0.37%) while the oxygenated sesquiterpenes were α-cadinol (0.59%) and farnesol (0.13%). The relative percentage of carvacrol (monoterpenoid phenol) was 0.13%. The major oxygenated hydrocarbons were decanal and hexadecanoic acid. Representative GC-MS chromatogram is given in Figure 1. In another study, volatile constituents of *Z. rhetsa* leaves and seeds analyzed by GC and GC-MS reported 118 compounds from the leaf oil and 77 compounds from the seed oil of the same plant[5]. Caryophyllene oxide (12.7%), β-caryophyllene (9.6%), β-copaene (5.3%) and spathulenol (3.3%) were the main components of the leaf oil, while, sabinene (66.3%), α-pinene (6.6%), β-pinene (6.3%) and terpinen-4-ol (3.5%) were the major components of the seed oil[5]. Sabinene (50%) was also reported as a major constituent of the essential oil from the same plant[3]. The chemical composition of the volatile oil of *Z. rhetsa* pericarp was similar to earlier reported study on the chemical analysis of seed coat[10]; the major compounds were terpinen-4-ol (32.1%), α-terpineol (8.2%), sabinene (8.1%), β-phellandrene (7.4%) and 2-undecanone (7.1%). The variations in the constituents are mainly due to the geographical source of the plant. Thus, analysis clearly showed that it is a different chemotype of species which is growing in the region and its pericarp is utilized in the fish curry and other culinary preparation due to its strong aroma. Results indicate that the essential oil obtained from *Z. rhetsa* grown in southern Karnataka is rich in β-pinene (10.4%), sabinene (16.50%) and terpinen-4-ol (25.43%). The total phenolic content of the pericarp was found to be 3.5±0.3 mg GAE/g which may be due to presence of carvacrol (phenolic monoterpenoid) and other compound. The sabinene, α-pinene and β-pinene content are much less in the plant grown in North-East region of India due to different environmental conditions[10].

Antioxidant Activity

The result of the antioxidant activity of the essential oil, fractions and terpinen-4-ol are reported in Table 3 and Figure 2. The results were compared with ascorbic acid, a well-known antioxidant molecule. The essential oil have demonstrated an interesting antioxidant power with IC_{50} value 7.5 ± 0.6 µg/mL, compared with ascorbic acid (5.1 ± 0.5 µg/mL). The IC_{50} value of the different fractions ranged from 51.3-84.0 µg/mL, while IC_{50} of terpinen-4-ol was 47.9 ± 2.0. It is interesting to mention that the fractions rich in sabinene and terpinen-4-ol were having more antioxidant capacity than the other fractions. The essential oil exhibited potent antioxidant activity due to the presence of different constituents like α-pinene, β-pinene, sabinene, γ-terpinene, terpinen-4-ol, α-terpineol, α-terpinolene[24] and γ-terpinene[24] and their synergistic effects. These results are in agreement with that obtained for the *Zanthoxylum* genus[25].

Antibacterial Activity

Table 3 reports the antibacterial activity of the tested oil against *S. aureus ATCC 6538a, E. coli ATCC 8739,* and *K. pneumonia*. The minimum inhibitory concentration (MIC) values for essential oil were ranged from 35 to 140 µg/mL (compared to ciprofloxacin, 0.73-1.46 µg/mL). The results indicate that the fractions were more active against the gram positive bacteria rather than gram negative due to their non-polar nature of the constituents. The isolated compounds (terpinen-4-ol) exhibited appreciable activity against all different bacteria studied. The strong antibacterial activity of essential oil is due to synergistic effects of active constituent (both polar and non polar) like 1-decene, *α*-terpinene, γ-terpinene, octanol, decanal[14], terpinen-4-ol, caryophyllene oxide, spathulenol, *α*-pinene, camphor and linalool[26-28].

Antidiarrheal activity

All mice in the negative control group showed diarrhea. Animals pretreated with higher dose of essential oil (200 µL/kg) showed 83.3% protection while at lower dose of 100 µL/kg the percent protection reduced to 67.3%. The positive standard loperamide (10 mg/kg,) showed 100% protection. As far as antidiarrheal activity of fractions is concerned it may be related to the α-pinene, β-pinene, α-terpineol and terpinen-4-ol content. In the earlier reported research on stem bark[12] the constituents responsible for antidiarrheal activity were not investigated. Results of present study indicate that the antidiarrheal activity of the oil is associated to the spasmolytic activity of active constituents of oil [α-pinene[29,30], β-pinene[29,30], and terpinen-4-ol (Table 5)] which reduce the peristaltic movement. Fraction F-3 and F-4 were less active than F-14 and F-15, which might be due to weak anticholinergic activity of their constituents, more investigation are required to establish the relationship.

Spasmolytic Activity

The essential oil showed weak nonspecific spasmolytic activity *in-vitro* (Table 5). The IC_{50} (µg/mL) of essential oil were 23.0±1.2 and 39.0±1.8 against contractions induced by acetylcholine ($2.5x10^{-7}$g/mL), and histamine ($3.0 x10^{-7}$g/mL), respectively. Essential oil did not produce any significant effect against contraction induced by nicotine. The fraction F-3, F-4, F-5, F-14 and F-15 and terpinen-4-ol exhibited appreciable anticholinergic activity. The IC_{50} (µg/mL) of fractions and terpinen-4-ol ranged from 40.0±3.5 to 70.0±4.3. The activity exhibited by essential oil was greater than the terpinen-4-ol (isolated compound) or fractions which might be due to the synergistic effect of the different constituent on activity. This study supports that the nonspecific spasmolytic activity of oil and anti-cholinergic effect of terpinen-4-ol (Table-5) are responsible for controlling the diarrhea and hyperactivity of intestine. The isolated fractions did not inhibit the contractions induced by nicotine or histamine.

CONCLUSION

This study indicates that essential oil of pericarp of *Z. rhetsa* have a significant antioxidant and antibacterial activities. The pericarp oil significantly protects the animal from castor oil induced diarrhea, which may be attributed due to non specific spasmolytic activity of oil and anti-cholinergic effect of the major component terpinen-4-ol. The antioxidant and antibacterial activities are due to terpinen-4-ol and various other constituents present in the oil. This plant can be further assessed for other active compounds and future therapeutic potential. Currently there is considerable interest in new natural antioxidants, antibacterial and antispasmodic agent to replace the synthetic ones that are used in foods and cosmetics. Identification of all the constituents from the plant source that are responsible for antioxidant activity requires further investigation, although it is obvious that constituents like polyphenols, tannins, reducing sugars, and proteins, which are present in the plants, might be responsible for synergistic activity.

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تحليل GC-MS والتقييم البيولوجي للزيت العطري من قشرة Zanthoxylum rhetsa (Roxb.) Dvm

راجشري راماكريشنا نايك[1]، اشوك كومار شاكيا[1]، نعمان عبد الكريم خلف[1]، سوسن أبو حمدة[2]، غالب علي عريقات[1]، أنور دياب مرقة[1]

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ملخص

تبين الدراسة الحالية التركيب الكيميائي الفاعل للزيت الطيار، والذي هو جزء واحد من المركبات الرئيسة، في نبات Zanthoxylum rhetsa (Roxb.) DC كمضاد للأكسدة وكمضاد للبكتيريا ومضاد للإسهال وكذلك مضاد للتشنج العضلي.

تم وصف مكونات الزيت الطيار بوساطة أجهزة .GC-FID,GC-MS. النشاطات الدوائية والبيولوجية للزيت، ومكوناته الرئيسة تم عملها داخل الجسم الحي في الفئران وفي المختبر.

المونوتيربينات (monoterpenes) الموجودة في الزيت الطيار هي: terpinen-4-ol (25.43%), sabinene (16.50%), β-pinene (10.4%),

α-terpineol (7.63%),

γ-terpinene فئق (terpinene−(%25.43)01−4−terpinen,(%terpinene−r(%25.43)01−4−pinen) وواحد من (5.64%), α-pinene (4.33%)

و linalool (3.25%)

تم تعيين القدرات المضادة للاكسدة للزيت الطيار، و terpinen-4- ol باستخدام مقياس الطيف لقياس كناس الجذور الحرة 2,2-dipheny-1- picrylhydrazyI (DPPH).

علاوة على ذلك، الزيت الطيار و terpinen-4- ol تظهر أو (تعطي) قدراً من مضاد الأكسدة، مضاد البكتيريا، مضاد الإسهال، وفعالية مضادة للتشنج غير انتقائية.

تقترح الدراسة أن المكون الفاعل (terpinen-4-ol) للنبات يمتلك جهد عال في علاج الإجهاد وأمراض الجهاز الهضمي.

الكلمات الدالة: Zanthoxylum rhetsa، مضاد الأكسدة، مضاد البكتيريا، مضاد التشنج العضلي.