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Original Article

Comparative Study for the Volatile Oil Constituents and Antimicrobial Activity of *Rhanterium epapposum* Oliv. Growing in Qassim, Saudi Arabia

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ABSTRACT

Background: *Rhanterium epapposum* is an herbaceous plant widely distributed in the Gulf region and used by Bedouins as antiseptic for wounds, skin infections and in gastrointestinal disturbances. **Aim:** The study aimed to compare the results obtained from volatile oil analysis of *R. epapposum* growing in Buraydah, Qassim with reported data of the same plant growing in Riyadh "Al-Majmaah" and Northern border region of Saudi Arabia. Both cold and hot extracts of the *R. epapposum* were used to find the best extraction method to be adopted as an antimicrobial agent. **Methods:** Volatile oils were distillated using Clevenger apparatus and analyzed by GC-MS. The plant powder was extracted by cold maceration and hot contentious extraction methods. Furthermore, antimicrobial activity was conducted using the agar diffusion method. **Results:** The hydro-distillation of *R. epapposum* growing in Qassim region yielded 0.5 % w/w of the total volatile oils. Moreover, forty-three compounds of 97.68% of the volatile oil components were identified while modephene, caryophyllene, linalyl acetate and epizonarene were the major components of volatile oils. Nevertheless, the volatile oils diversity and concentrations were found to be different in *R. epapposum* growing in Buraydah, as compared with the plant growing in Riyadh and the northern border region of Saudi Arabia which almost due to the differences in the environmental condition. Among all extracts, ethyl acetate hot extract showed the best inhibition to bacterial strains while fugal strain *Candida albicans* growth was better inhibited by hot *n*-hexane extract. **Conclusion:** Volatile oils were active against all microbial strains. Hot extracts were more active against bacterial strains while the opposite effects were found against *Candida albicans* which was generally inhibited by the cold extracts.

Key words: *Rhanterium epapposum*, Essential Oils, Antimicrobial activity, Comparative study, Hot extraction. Cold extraction.

INTRODUCTION

Rhanterium epapposum "Al-Arfaj" is a small leafy plant usually grows and adapted to dry climate and widely spread in deserts, villages, oases, mountains, valleys and plains.[1] Therefore, this plant is widely distributed in North African countries[1] and Arab Peninsula particularly, Saudi Arabia, Kuwait and Arab United of Emirates deserts and known by local people as a grazing herb for sheep, goats and camels.[1-4] *R. epapposum* is known for its insecticide effect[5] and used in traditional medicine for the treatment of gastrointestinal disorders and skin infections.[6]

The volatile oil constituents of *R. epapposum* collected from many eastern countries such as Saudi Arabia[7,8] and Iran[9] have been previously measured. The common essential oil constituents in *R. epapposum* were the oxygenated monoterpenes such as Limonene, Linalool, geraniol and α-Terpineol,[7] non-oxygenated terpenes such as α-phellandrene and sesquiterpenoid essential oils such as α-cadinol,[7,9] Sterols and triterpenes, carotenoids, alkaloids, coumarins, tannins, flavonoids, polyuronides and saponins have also been identified in petroleum ether, methanol and aqueous extracts of *R. epapposum*. The antimicrobial[10] and wound healing activities[11] for the extracts as well as volatile oils of *R. epapposum* have been reported. *R. epapposum* honeybees (Spring Lena honey) has been shown a strong inhibition toward different microbial strains such as *Staphylococcus aureus* and *Streptococcus mutans* Gram-positive bacteria in addition to Gram-negative strains such as *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*.[12] *R. epapposum* ethaol extract was reported to have

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antioxidant activity which attributed to its total phenolic constituents.[13] Also, the anti-inflammatory effect of *R. epapposum* has been measured in addition to their total flavonoids and phenolic constituents. The study revealed that this plant extract could be used for treatment of rheumatism and other inflammatory disorders.[14] Furthermore, *R. epapposum* extract was exhibit cytotoxic effect against leukemia CCRF-CEM cell line.[15]

The present study was planned to investigate the volatile oil constituents and design the best extraction method for the maximal antimicrobial activity of *R. epapposum* growing in Buraydah, Qassim region. In addition, the volatile oil constituents have been compared with the recently reported data for the volatile oils isolated from the same plant growing in Riyadh and the northern border region.

MATERIALS AND METHODS

Plant materials

The plant was collected in April 2017, during the flowering stage from central Buraydah city, in Qassim and was identified as *Rhanterium epapposum* family *Asteraceae* by the agricultural experimental station in Unaizah. A voucher specimen of the plant under a number of 77 is deposited at herbarium of College of Pharmacy, Qassim University. The whole plant materials were spread in an open-air area until completely dry; then the dried plant materials were ground to a coarse powder before use.

Hydro-distillation of volatile oils

An amount of 250 g of coarse dried plant material was placed in a 500 ml round bottom flask connected with a Clevenger distillation apparatus;[16] the distilled water containing volatile oils (distillate) was collected and extracted three times with chloroform. The chloroform layer containing volatile oils was dried with anhydrous sodium sulfate to remove all water and then the solvent was evaporated with a rotary evaporator under reduced pressure at room temperature to get the volatile oil.

Gas chromatography-mass spectroscopy analysis (GC-MS)

The GC-MS analysis was carried out on a Trace 1300 GC, Tsq DUO Triple Quadrupole MS with a column TG 5MS (30m × 0.25mm, 0.25µm). Helium was used as a carrier gas at a flow rate of 1.2 ml/min. Split/Splitless (S/SL) injector was used with 250°C injector temperature. 1.0µl sample injection volume was utilized. Ion source temperature was maintained at 200°C. The oven temperature was programmed initially at 40°C for 4 min, then programmed to increase to 250°C at a rate of 10°C/min ending with a 5 min isothermal at 280°C. Total run time was 27.0 min. The MS transfer line was maintained at a temperature of 250°C. TSQ DUO Triple Quadrupole MS detector (mass range from m/z 45 to 600) was used for analysis.

The mass spectra of the components were matched with the data available in the National Institute of Standards and Technology (NIST) library (Table 1).

Extraction Method
Hot extraction

An amount of 200 g of the dried plant material were extracted by continuous Soxhlet extraction apparatus[17] using *n*-hexane, chloroform, ethyl acetate and ethanol, respectively. After 10 refluxes, each extract was dried under vacuum at 40°C and the extractive values were calculated as grams of dried extracts obtained from hot extraction procedure per 100 gm of the dried plant powders (Table 2).

Table1: The Volatile Oil Contents of *Rhanterium epapposum* Olive herb.

No	Name	RT	Relative Percentage (%)[RP]
1	Limonene*	10.682	1.64
2	Linalool*	11.629	3.80
3	Linalyl acetate	12.031	8.46
4	α-Terpinyl acetate	13.015	0.82
5	Terpinen-4-ol*	13.191	0.37
6	L-α-Terpineol*	13.444	3.39
7	Nerol*	13.889	2.23
8	Geraniol*	14.341	3.20
9	Citronellyl acetate*	14.553	0.43
10	7-α-[H]-Silphiperfol-5-ene	15.393	1.81
11	7-epi-Silphiperfol-5-ene	15.660	1.74
12	Modephene	16.216	10.51
13	Methyl eugenol*	16.337	1.16
14	Longifolene	16.560	3.44
15	Caryophyllene*	16.752	8.59
16	9,11-Dodecadien-1-ol	16.904	0.77
17	cis-7-Tetradecen-1-ol	17.005	1.18
18	1,5,9,9-tetramethyl-1,4,7,-Cycloundecatriene	17.133	0.69
19	Aromandendrene	17.228	0.53
20	Aristolene	17.315	0.54
21	Guaia-1(10),11-diene	17.545	2.41
22	(2R,8R,8aS)-8,8a-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,7,8,8a-hexahydronaphthalene	17.754	1.48
23	ϒ-Cadinene*	17.858	1.64
24	α-Cedrene*	17.933	1.06
25	Nerolidol	18.334	0.80
26	Ledene oxide	18.739	1.50
27	Caryophyllene oxide*	18.779	2.07
28	Tau-Cadinol acetate	18.914	0.65
29	Ethanone, 1-[2-[2-methyl-2-(5-methyl-2-furanyl) propyl] cyclopropyl]	19.133	2.75
30	Acetic acid, 3-hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl ester	19.184	0.71
31	α-Cadinol *	19.383	6.35
32	Epizonarene	19.437	5.43
33	3,5,11-Eudesmatriene	19.585	3.58
34	(1S,4aS,7R,8aS)-1,4a-Dimethyl-7-(prop-1-en-2-yl)decahydronaphthalen-1-ol	19.690	2.41
35	Calarene epoxide	19.734	0.84
36	β-Eudesmol*	19.973	1.40
37	α-Vetivol	20.094	0.98
38	Valerenol	20.270	0.93
39	Vetivenic Acid	20.563	1.44
40	(8S,14)-Cedran-diol	20.644	1.06
41	Froggatt ether	20.803	1.22
42	Isolongifolan-8-ol	21.022	0.33
43	6,10,14-Trimethylpentadecan-2-one	21.390	1.34
	Total		97.68%

RT= Retention time on the column in minutes.

[RP]Percentage was calculated according to the relative area (peak area relative to the total peaks area).

*Essential oils detected in *R. epapposum* growing in Riyadh (Al-Majmaah) and/or northern border region of Saudi Arabia.

Table 2: Extractive values and antimicrobial effects of *R. epapposum* extracts and volatile oils represented as inhibition zone diameter (IZD) in mm.

Items	n-Hexane extract		Chloroform extract		Ethyl acetate extract		Ethanol extract		Volatile oils	Positive control
Extractive values*	Cold 1.2%	Hot 0.7%	Cold 2.8%	Hot 1.39%	Cold 1.7%	Hot 0.7%	Cold 1.8%	Hot 0.65%		
Staphylococcus aureus	n	n	19	21	19	25	12	14	16	Erythromycin 26
E.coli	n	12	10	10	10	16	13	12	12	Amoxicillin 13
Candida albicans	15	26	20	12	19	9	10	n	19	Clotrimazole 32

n= inhibition zone not detected

*Microbial experiments were carried out in triplicate and mean were calculated to the nearest mm diameter.

*Extractive values were calculated as gram of extracts per 100 gm of the dried plant materials.

Cold Extraction

An amount of 200 g of dried plant materials was transferred to a 1000 ml conical flask. 500 ml of *n*-hexane was used to extract the plant constituents at room temperature.[18] After 24 h stirring, the extract was filtered and dried under vacuum at room temperature. The residue of the plant materials left after removal of *n*-hexane extract was subjected to extraction by chloroform, ethyl acetate and ethanol, in sequence using the same previous manner used in *n*-hexane extract. The extractive values of cold extracts were calculated as grams of dried extracts obtained from the cold extraction procedure per 100 gm of the dried plant powders (Table 2).

Determination of antimicrobial activity

The antimicrobial screening of *R. epapposum* volatile oils, as well as cold and hot extracts, was performed using agar diffusion method described by Cooper and Woodman.[19] The microbial suspensions of the gram-negative *Escherichia coli*, gram-positive *Staphylococcus aureus and Candida albicans* yeast were prepared, incubated for few minutes' till they showed turbidity/growth comparable to 0.5 McFarland tube as a reference. Sterile swaps were smeared on the agar surface, incubated for few minutes. Then 50µl of the test sample was micropipetted to the designed cups. Positive control drug disc (10µg/ml amoxycillin, gentamycin and clarimazole) were placed onto the top of the inoculated agar plate which were then incubated. Plates were then incubated (Sheldon Manufacturing INC., USA) for 24-72 h depending on the growth rate of each microbial strain and were checked every day and inhibition zone diameters (IZD) measured to the nearest mm.[20-22]

RESULTS AND DISCUSSION

Volatile oil constituents of *R.epapposum* growing in Qassim region

R. epapposum is a wildly herbaceous plant, wide-distributed in deserts of Saudi Arabia, particularly the middle and northern region of the kingdom. The GC-MS analysis of the volatile oils of *R. epapposum* growing in Qassim yielded 43 volatile oil constituents as it is shown in Table 1. These volatile constituents have been previously isolated from a huge number of aromatic plants. Oxygenated and non-oxygenated monoterpene and sesquiterpene hydrocarbons were represented in the volatile oil constituents of *R. epapposum.* Limonene was the only non-oxygenated monoterpene compound with a percentage of 1.64 % of total volatile oil amount. On the other hand, eight oxygenated monoterpene compounds were detected which are Linalool, Linalyl acetate, α-Terpinyl acetate, Terpinen-4-ol, L-α-Terpineol, Nerol, Geraniol and Citronellyl acetate; they collectively represent 22.7 % of the total *R. epapposum* volatile oils. In addition, 14 non-oxygenated sesquiterpene compounds were detected in the volatile oil which was evaluated to be 43.5 % of the total volatile oils (Table 1). Out of the forty-three volatile oil compounds, there are 18 compounds of oxygenated sesquiterpenes which are represented by 28 % of the total volatile oil. The GC-MS analysis showed that modephene, caryophyllene, linalyl acetate and epizonarene were the major compounds among all other volatile constituents of *R. epapposum* growing in Qassim, with a percentage of 10.51 %, 8.59 %, 8.46 % and 5.43 %, respectively (Table 1).

The GC-MS analysis results were used to investigate the variation in the volatile oils production and diversity in the *R. epapposum* plant according to their climate growing area. Comparing the present results with the most recent reported data for the volatile oil constituents of *R. epapposu*m growing in Al-Majmaah region "Riyadh" (165 km away from Buraydah, the place where the *R. epapposu*m plant was collected and used in the current study)[8] and northern border region of Saudi Arabia,[7] indicates that the difference is marked not only in the concentration of particular compounds but also in their diversity, for instance, out of 43 volatile components of *R. epapposum* growing in Buraydah (Qassim), only 14 compounds were present in the same plant growing in Riyadh (Al-Majmaah) and/or northern border region. Furthermore, the concentrations of these 14 compounds were completely different from the *R. epapposum* volatile oil compounds collected from those places.[7,8] In addition, the most abundant constituents of the volatile oils of *R. epapposum* growing on Qassim were not detected during the analysis of volatile oils of *R. epapposum* growing in Riyadh (Al-Majmaah) and the northern border region. For instance, modephene (10.51%), Linalyl acetate (8.46%) and epizonarene (5.43%) that have been identified in large proportion in *R. epapposum* growing on Qassim, were not identified in the analysis of volatile oils of *R. epapposum* growing in Riyadh and northern border region (Table 1).[7,8]

Extractive values and antimicrobial activity of *R. epapposum* extracts

The extractive values obtained from both cold and hot extraction methods are summarized in Table 2. Among all solvents, chloroform produced the highest amount of extract 2.8 gm (cold) and 1.39 gm (hot) per 100 gram of the dried plant powder. The extractive values also showed that the extractive cold materials were almost Twice the materials obtained from hot extraction method (Table 2).

The antimicrobial activity of *R. epapposum*, as well as its honey, is well established literatures reported previously.[8,10,12] In addition, *R. epapposum* has a wound healing activity that may be attributed to its antimicrobial effect.[11] Therefore, we extracted *R. epapposum* herb with different solvents and by two different methods (cold and hot methods) to investigate the best solvent and better method to extract the antimicrobial constituents of *R. epapposum*. In this regard, three microbial stains represent the Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*E. coli*) in addition to fungal strain (*Candida albicans*) were used to determine the antimicrobial effect of the *R. epapposum* fractions (Table 2).

The result shown in Table 2 indicates that the *n*-hexane fraction was active against *Candida albicans* fungus strain with 15 mm and 25 mm inhibi-

tion zone diameter (IZD) for both cold and hot extract respectively. In addition, *n*-hexane hot extract was active against Gram-negative *E. coli* while cold extract was inactive against the same strain. It is noteworthy that both *n*-hexane extracts were inactive against Gram-negative *Staph aureus*. On the other hand, chloroform extracts were active against all microbial strains. Chloroform cold and hot extracts were almost having the same activity in both bacterial strains (19 mm and 21 mm IZD for cold and hot extract against *Staph. aureus,* respectively and 10 mm IZD for both chloroform extracts against *E. coli*. Chlorofolm cold extract was more active against *Candida albicans* fungal strain which showed 20 mm IZD versus 12 mm IZD in case of hot chloroform extract. The ethyl acetate hot extract was more active for both bacterial strains where the activity was nearly twice less in case of *Candida albicans.* The inhibition zone diameter for hot and cold ethyl acetate extracts indicates as follows (18 mm IZD and 24 mm IZD against *Staph. aureus,* 10 mm IZD and 16 mm IZD against *E. coli* and 19 mm IZD and 9 mm IZD against *Candida albicans* in cold and hot ethyl acetate extracts, respectively). The antibacterial activity of cold and hot ethanol extracts were almost the same for *Staph. aureus* and *E. coli* strains except in case of *Candida albicans* which was inhibited by cold extract (10 mm IZD); and no effect for hot extract (Table 2). The antimicrobial effects of *R. epapposum* volatile oils were measured and the results indicated that volatile oils were active against both bacterial and fungal strains with a similar average to plant extracts (Table 2). Regarding positive controls, ethyl acetate hot extract was nearly similar in activity with erythromycin and more active than amoxicillin in case of *Staph aureus* and *E. coli* bacterial strains, respectively (Table 2). On the other hand, both hot and cold extracts and volatile oil obtained from the plant were less active than clotrimazole (positive control) against *Candida albicans* fungal strain (Table 2).

CONCLUSION

The GC-MS analysis indicates that *R. epapposum* contained with a wide variety of volatile oils include oxygenated and non-oxygenated monoterpenes and sesquiterpenes. The results also indicate that these volatile oils diversity and concentrations are highly affected by the environmental climate. The results also generally indicate that hot extraction method is preferred for the antibacterial effect of *R. epapposum.* Oppositely, the good finding with cold extract was measured against fungal Candida *albicans* except for *n*-hexane extract. Further studies are planned to isolate the hot ethyl acetate extract constituents which have the best antibacterial activity among all extracts.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to disclose.

ABBREVIATIONS

GC-MS: Gas chromatography Mass Spectroscopy; ***R. epapposum*:** *Rhanterium epapposum*; **NIST:** National Institute of Standards and Technology; **IZD:** Inhibition zone diameters; ***Staph. aureus*:** *Staphylococcus aureus*; *E. coli: Escherichia coli.*

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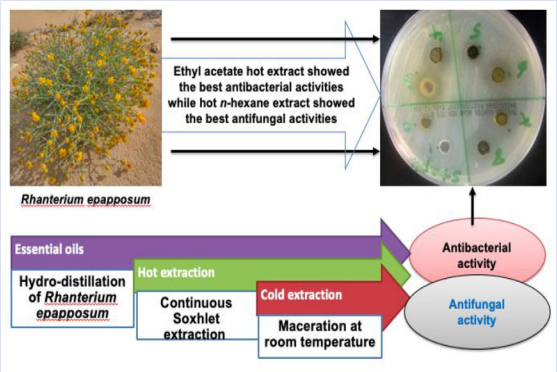
GRAPHICAL ABSTRACT



SUMMARY

- *R. epapposum* used in folk medicine as antiseptic for wounds, skin infections and in gastrointestinal disturbances.
- *R. epapposum* growing in Qassim region yielded 0.5 % w/w of the volatile oils.
- Forty-three compounds representing 97.68% of the volatile oil components were identified by GC-MS analysis.
- Volatile oils diversity and concentrations was found to be different in *R. epapposum* growing in Buraydah, as compared with the plant growing in Riyadh and Northern border region of Saudi Arabia.
- Both cold and hot extracts of the *R. epapposum* were used to find the best extraction method to be adopted as an antimicrobial agent.
- Hot extracts were more active against bacterial strains while the opposite effects were found against *Candida albicans* fungal strain.