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Chemical Composition, Anti-inflammatory, Analgesic, Antipyretic, Myorelaxant, Antibacterial and Antifungal activity of *Rabdosia rugosus* Wall. (Syn. *Plectranthus rugosus* Wall.)

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ABSTRACT

For the present investigation *Rabdosia rugosus* Wall. Syn. *Plectranthus rugosus* Wall. was collected from Pancheshwar, Uttarakhand on the way to Badrinath. The GC and GC-MS analysis, revealed the presence of more than forty compounds out of which 35 compounds were identified amounting to 97.3% of the total oil. The essential oil of *R. rugosus* was rich in sesquiterpinoids (~90%) and was poor in monoterpenoids (8.1%). α-bisabolol (41.9%) was the major constituent of the oil and the other identified major compounds were germacrene-D (9.7%), β-caryophyllene (7.6%), dehydroabietane (5.2%), *ar*-curcumene (5.0), *trans*-ferruginol (3.3%), α-cadinol (3.2%), T-muurolol (2.3%), *p*-Cymene (3.2%) and γ-terpinene (2.0%). The essential oil of *Rabdosia rugosus* showed insignificant anti-inflammatory and analgesic activity but shows significant antipyretic, myorelaxant and antimicrobial activity.

KEYWORDS: *Rabdosia rugosus*, α-bisabolol, anti-inflammatory activity, myorelaxant activity, antimicrobial activity

INTRODUCTION

The genus Plectranthus consists of about 300 species, belongs to the family Lamiaceae and is widely distributed in Africa, Asia, Australia and some Pacific islands. Many species of Plectranthus are economic and medicinal interest. Several species are cultivated for their edible tubers or as essential oil crops and others are used as food flavouring or fragrance. Some others are also used for medicinal purposes to treat vomiting and nausea, ear infections, respiratory diseases, toothache, headache, sores, and burns or as an antiseptic, purgative, antimicrobial and stimulant [1-5].

Rabdosia rugosus Wall. (Syn. *Plectranthus rugosus* Wall.), an aromatic shrub is found in Himalayas to Nepal, southeast Arabia (Oman), Afghanistan, Pakistan, and southwest China, very commonly growing on dry mountain slopes at lower altitudes. Plants are much branched, to 1.5 m tall aromatic shrubs. Stems are erect with rather slender quadrangular branches, leafy, with an indumentum of small stellate dendroid hairs. Leaves are opposite with 2-10 mm long petioles. Leaf lamina is ovate- elliptic, rugose, reticulate and usually dark green on adaxial side and white tomentose with dense stellate trichomes on abaxial side. Flowers are in axillary racemes on terminal leafy panicles. Flowers are white-purplish and pedicellate with up to 6 mm long erect pedicel. Calyx in flower is 2-3.5 mm, indistinctly bilabiate, obliquely campanulate, with simple and/or branched hairs of varying length and density and usually numerous oil globules; calyx enlarging in fruiting stage up to 6 mm. Corolla are bilabiate, upper lip white and spotted with purple, lower lip boat-shaped, entire. Fruit is schizocarp of pale brown to dark brown nutlets. The plant flowers from March-October. According to R. R. Stewart, it is one of the commonest shrubs in the west Himalayas and a good honey source. It is usually a plant of dry rocky slopes, where it can be a dominant species in the community [6,7].

The leaf and inflorescence essential oils of *P. rugosus* Wall. and *P. incanus* L. from Uttarakhand, India, were dominated by sesquiterpene hydrocarbons. β-caryophyllene (36.2%, 29.8%), germacrene D (25.2%, 28.2%) and α-humulene (6.6%, 8.6%) were analysed as the major constituents [8]. Sesquiterpene hydrocarbons rich essential oil of *R. rugosus* also reported by Tiwari *et al.*, [9]. β-caryophyllene (38.4%) and germacrene D (23.8%) were the major identified components. The essential oil from *P. rugosus* growing in the Jammu and Kashmir state was reported to yield 0.17 % oil. Spatulenol, germacene D and β-caryophyllene were identified as the major constituents among the twenty three compounds. The essential oil of *Plectranthus rugosus* was also reported to have antifungal activity against *Microsporum canis* and *Fusarum solani* [10]. Weyerstahl *et al.*, [11] reported caryophyllene, germacrene-D, α-phellandrene, α-pinene, caryophyllene oxide, α-cadinol, δ-cadinene, limonene, β-phellandrene, myrcene, and p-cymene as major constituents in the essential oil of *P. rugosus*. Razdan *et al.*, [12] isolated and identified two new triterpenoid acids (plectranthoic acid A and plectranthoic acid B), sitosterol and three new pentacyclic triterpenoids (plectranthoic acid, acetylplectranthoic acid and plectranthadiol) from *P. Rugosus* [12]. Hence, the present study analyzed the chemical composition of the essential oil and evaluated its in vitro anti-inflammatory, antinociceptive, antipyretic, myorelaxant, antibacterial and antifungal activities.

MATERIAL AND METHODS

Plant Source

Rabdosia rugosus Wall. Syn. *Plectranthus rugosus* Wall. was collected from Pancheshwar, Uttarakhand on the way to Badrinath, India and was identified by plant taxonomist Dr. D.S Rawat. The specimens have been deposited in the Department of Chemistry, Pantnagar for future reference.

Isolation of Essential Oil and GC/MS Analysis

Essential oil extraction was carried out by following standard methods [13] as explained previously [30]. GC–MS analysis was done [14] as explained before [30].

Experimental Animals

Animals {Swiss albino mice (R)} were procured from Lab animal division, Central Drug Research Institute, Lucknow, India. The mice were divided into four groups of six mice each for the experiments. They were housed in standard cages at a constant temperature of 22±1°C, relative humidity 55±5% with 12 hr light–dark cycle (08:00 to 20:00) for one week at least before the experiment. The experimental protocol was approved by the Committee on Animal Research, (ethical committee) with Registration No. 330/CPCSEA. All tests were conducted under the guidelines of the ethical committee for the study.

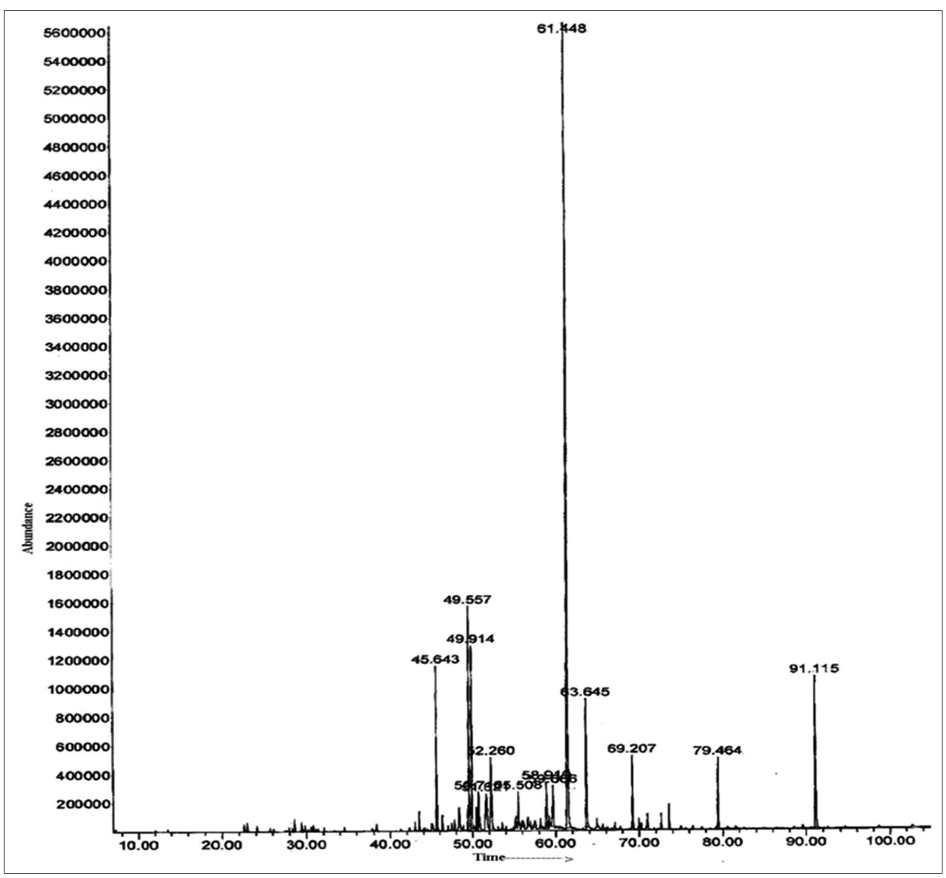

Figure 1: Gas Chromatogram of *Rabdosia rugosus* Wall. essential oil

Tabel 1: Essential oil composition (%) of *Rabdosia rugosus* Wall. (Syn. *Plectranthus rugosus* Wall.)

Compounds	RI	Present investigation	Weyersthal *et al.* (1983)	Tewari *et al.* (2008)	Padalia and Verma (2011)		Muhammad *et al.* (2012)
α-pinene	939	t	3.1	1.2	0.6	t	0.06
camphene	946	-	0.03	-	-	-	-
sabinene	978	-	0.2	-	0.2	t	-
β-pinene	982	-	0.1	-	1.2	0.1	-
myrcene	992	0.4	2.1	-	0.4	t	4.0
α-phellandrene	1002	-	4.6	-	-	-	-
3- carene	1006	-	0.7	-	-	-	4.8
α-terpinene	1016	-	0.04	-	t	0.4	-
p-cymene	1028	3.2	2.1	3.6	2.2	1.1	3.0
β-phellandrene	1031	-	2.3	-	-	-	-
limonene	1032	1.0	2.4	2.7	0.5	t	2.7
cis-β-ocimene	1032	-	0.2	-	-	-	-
1,8-cineole	1035	-	-	-	2.4	4.6	-
trans-β-ocimene	1044	-	1.0	-	-	-	-
γ-terpinene	1062	2.0	0.2	2.8	0.4	0.3	2.0
terpinolene	1087	0.1	1.0	0.7	1.4	2.4	-
linalool	1096	0.1	0.9	0.3	1.2	4.2	-
1-nonene-3-ol	1102	-	0.7	-	-	-	-
thujone	1106	-	-	-	-	-	-
cis-p-menth-2-en-1-ol	1124	-	-	-	t	t	-
trans-p-menth-2-en-1-ol	1142	-	-	-	t	t	-
borneol	1165	-	-	-	t	0.2	-
terpinen-4-ol	1178	0.2	0.7	-	0.9	1.0	-
naphthalene	1179	-	-	-	-		1.70
α-terpineol	1190	1.2	-	-	2.4	1.4	-
piperitone epoxide	1250	0.8	-		-	-	-
bornyl acetate	1284	-	-	-	t	t	-
α-cubebene	1351	-	-	-	-	-	0.36
α-copaene	1375	0.3	0.4	t	t	-	0.9
β-patchoulene	1380	-	-	t	-	-	-
β-bourbonene	1387	-	0.3	-	-	-	-
β-cubebene	1391	t	0.4	0.1	–	-	-
β-elemene	1393	0.1	-	0.6	–	-	-
β-caryophyllene	1418	7.6	22.0	38.4	36.2	29.8	10.6
thoujopsene	1429	-	-	-	-	-	0.5
β-gurjunene	1434	t	-	0.1	t	0.4	-
α-humulene	1455	0.2	1.6	1.7	6.6	8.6	-
(E)-β-farnesene	1460	1.0	-	-	2.3	3.8	-
γ-muurolene	1477	0.2	1.4	0.6	0.1	t	-
germacrene D	1482	9.7	7.5	23.8	25.2	28.2	20.0
ar-curcumene	1483	5.0	0.2	-	-	-	-
epi-cubebol	1493	0.5	-	0.8	1.0	t	-
α-selinene	1494	t	-	0.8	0.1	t	-
α-murrolene	1499	t	1.0	0.1	-	0.3	-
bicyclogermacrene		-	0.8	-	-	-	-
γ-cadinene	1514	0.5	1.1	1.6	-	t	-
cubebol	1518	t	-	-	0.3	0.1	-
δ-cadinene	1525	4.8	2.8	0.1	1.3	0.6	-
ledol	1542	-	-	-	-	-	1.8
β-caryophyllene oxide	1556	-	3.1	-	-	-	7.0
germacrene D-4-ol	1576	0.6	-	1.3	1.4	1.2	-
spathulenol	1578	0.5	-	3.2	1.2	0.2	21.0
caryophyllene oxide	1580	1.3	3.1	0.3	1.3	t	-
humulene epoxide II	1609	t	-	-	0.2	0.1	-
α-cardinol	1611	-	-		-	-	2.0
T-muurolol	1633	2.5	-	-	-	-	-
T-cadinol		-	1.2	-	-	-	-
epi-α-Cadinol	1642	1.0	-	0.9	0.3	0.1	-
torreyol	1645	-	1.3	-	-	-	-
α-cadinol	1653	3.2	3.1	2.2	t	0.4	-
β-bisabolol	1656	-	-	-	-	-	0.2
α-bisabolol	1683	41.9	-	-	-	-	0.2
phytol	1949	-	-	-	-	-	0.3
dehydroabietane		3.3	-	-	-	-	-

(Contd...)

Tabel 1: *(Continued)*

Compounds	RI	Present investigation	Weyersthal *et al.* (1983)	Tewari *et al.* (2008)	Padalia and Verma (2011)		Muhammad *et al.* (2012)
trans-ferruginol	2325	5.2	-	-	-	-	-
Total		97.3	73.67	87.9	91.3	89.5	83.12

t- traces (<0.1%)

Table 2: Acute anti-inflammatory activity of essential oils of *Rabdosia rugosus* (Mean±SE, n=6)

Group	Treatments	Dose (mg/kg)	Change in paw thickness			% inhibition	
			0 hrs	4 hrs	24 hrs	4 hrs	24 hrs
1	Control	0.20 ml	2.40±0.02	2.33±0.01	2.32±0.01	2.92	3.06
2	Ibuprofen	40	2.34±0.01	1.73±0.02[a]	1.47±0.02[a]	25.77	37.19
3	RREO	50	2.32±0.01	2.29±0.02	2.17±0.01[ab]	1.44	6.61
4		100	2.32±0.01	2.29±0.01	2.16±0.04[ab]	1.44	6.97

(one way ANOVA followed by dunetts multiple comparison test); [a]=significant ($p<0.05$) as compared to control; [b]=significant ($p<0.05$) as compared to drug; RREO=*Rabdosia rugosus* essential oil

Anti-Inflammatory, Analgesic Activity and Antipyretic Activity

Anti-inflammatory activity [15], carrageenan-induced mice paw edema [16], formaldehyde induced paw edema [17] and analgesic activity (Acetic acid-induced writhing response) [18] were estimated by following standard methods as explained [30]. Hyper analgesic reaction in mice was performed by hot plate method [19] and antipyretic activity as described by Rao *et al*., [20].

Toxicity

The acute toxicity test in mice and rats was carried out as explained previously [30].

Effect of Essential Oil on Isolated Duodenum Smooth Muscles of Wistar Rats

Effect of essential oil on isolated duodenum smooth muscles of Wistar rats was analysed by the method described by Gangwar *et al*., [31].

Preparation of Essential Oil and Drugs

The test solutions of *R. rugosus* essential oil (RREO) were freshly prepared along with acetylcholine (Ach), adrenaline, atropine and propanolol in desired concentrations and were used for the experiments. The tissues were allowed to show the maximum response of adrenaline, which took about 1–1.5 minutes. Then test oils as used in previous concentrations were repeated to compare the effect with adrenaline. Then 100 μg of propanolol was added to the organ bath. The tissues were allowed to show the maximum response of propanolol. After the dose of propanolol, adrenaline and essential oil were repeated in the same order.

Antibacterial and Fungicidal Activities

Antibacterial, one gram negative (*Salmonella enterica enterica*) and one gram positive (*Staphyllococcus aureus*) [21] and Fungicidal (*Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium oxysporum)* [22] activities were estimated by following standard methods as explained before [30].

Statistical Analysis

Data were expressed as Mean±S.E. Results were analysed using one way ANOVA followed by dunetts multiple comparison test and $p<0.05$ was considered to be statically significant.

RESULTS AND DISCUSSION

Chemical composition

For the present investigation *R. rugosus* was collected from Pancheshwar, Uttarakhand on the way to Badrinath. The GC and GC-MS analysis, revealed the presence of more than 40 compounds out of which 35 compounds were identified amounting to 97.3% of the total oil (Figure 1). The oil was poor in monoterpenoids (8.2%). The monoterpens identified included p-Cymene (3.2%), γ-terpinene (2.0%), α-terpineol (1.2%), limonene (1.0%), myrcene (0.4%), terpinen-4-ol (0.2%), terpinolene (0.1%), and linalool (0.1%). α- pinene could be detected in traces only.

The essential oil of *R. rugosus* was rich in sesquiterpinoids (~90%). The identified sesquiterpene hydrocarbons were germacrene-D (9.7%), β-caryophyllene (7.6%), *ar*-curcumene (5.0%), (E)-β-farnesene (1.0%), α-copaene (0.3%), γ-muurolene (0.2%), humulene (0.2%), β-Elemene (0.1%), β-cubebene (t) and β-gurjunene (t). The identified oxygenated sesquiterpinoids in the essential oil included α-bisabolol (41.9%), α-cadinol (3.2%), T-muurolol (2.5%), epi-α-cadinol (1.0%), germacrene D-4-ol (0.6%), spathulenol (0.5%), epi-cubebol (0.5%), cubebol (t) and humulene epoxide (t). We could identify two diterpinods dehydroabietane (5.2%) and *trans*-ferruginol (3.3%) which were not earlier identified from this plant (Table 1). The other finding is the presence of large amount α- bisabolol (41.9%) in the oil which was otherwise been reported in low amounts in the earlier investigations.

The essential oil of *R. rugosus* (Syn. *Plectranthus rugosus*) has earlier been reported from Uttarakhand by Tewari *et al*., [9] and Padalia and Verma [8] and from Jammu by Weyersthal *et al*., [11] and Muhammad *et al*. [10]. Weyersthal *et al*., [11] reported caryophyllene (22.0%) as the major constituent besides germacrene-D (7.5%), α-pinene (3.1%), myrcene (2.1%), α-phellandrene (4.6%), limonene (2.4%), β- phellandrene (2.3%), *trans*-β-ocimene (1.0%), p-cymene (2.1%), δ-cadinene (2.8%), γ-cadinene (1.1%), caryophyllene oxide (3.1%), α-cadinol (3.1%) and humulene (1.6%). Tewari *et al*., [9] identified twenty-five components which constituted 87.9% of the total oil. β-caryophyllene (38.4%), germacrene D (23.8%), spathulenol (3.2%) and α-cadinol (2.2%) were the reported major sesquiterpene hydrocarbons. Monoterpenes hydrocarbons *p*-cymene (3.6%), γ-terpinene (2.8%) and limonene (2.7%) were the other identified components in the essential oil of *R. rugosus*. In our investigation α-bisabolol (41.91 %) the major constituent of the investigated oil which was reported in low quantity (0.2%) by Irshad *et al*., [23] but absent in the others reports. β-caryophyllene (7.53%) was present in less quantity in our finding as compared to 38.4% reported by Tewari *et al*. (2008). Germacrene-D a major constituent in earlier studies 25.2% and 28.2% [8], 23.8% [9] and 20.0% [10], was found to be only 9.76%. *ar*-curcumene (7.59%) was not reported in earlier studies. Spathulenol are also found to be absent in our collection though reported in earlier studies. We could identify two diterpenes dehydroabiatene and *trans*-ferruginol which were not reported in earlier studies. The findings are significant in view of chemodiversity in Rabdosia (syn. Plectranthus) species growing in Himalayan region. Detailed comparative analysis of essential oil of *Rabdosia rugosus* in present and earlier studies has been represented in Table 1.

Anti-Inflammatory, Analgesic and Antipyretic Activity

The anti-inflammatory effects of the essential oil of *R. rugosus* (RREO) on carrageenan induced edema in the mice right hind paw are presented in Table 2. There was a gradual increase in edema paw volume of mice in the control and RREO. However, in the ibuprofen treated group a significant reduction (37.19%) in edema was observed at 24th hr. The inhibitory effect of the RREO recorded with a dose level of 50 and 100 mg/kg b. wt. in 24 h were 6.61% and 6.97%, respectively. RREO could not produce significant reduction in paw volume at doses of 50 and 100 mg/kg b. wt. as compared to the control. In sub-acute anti-inflammatory activity, where arthritis was induced by formaldehyde injection on day zero and the samples were administered orally daily for 10 days. During the investigation the RREO was also found to be insignificant comparatively, ibuprofen (Table 3).

For the determination of centrally acting analgesics, the hot plate test was useful [24] which are known to elevate the pain threshold of mice towards heat. The less reaction time shown by the mice treated with RREO suggests that it is not an effective against centrally acting analgesic. (Table 4). Data recorded in Table 5 on the acetic acid-induced writhing responses in mice are presenting of no analgesic activity of RREO. There was no significant effect of RREO at 50 and 100 mg/kg. b.wt. in decreasing writhing

Table 3: Effect of essential oil of *Rabdosia rugosus* on formalin induced sub acute inflammation (Mean±SE, n=6)

Groups	Treatments	Dose (mg/kg)	Volume of inflammation										
			Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
1	Control	0.2 ml	2.13±0.02	2.27±0.04	2.36±0.03	2.52±0.04	2.38±0.03	2.30±0.02	2.30±0.02	2.28±0.01	2.27±0.01	2.21±0.01	2.26±0.01
2	Ibuprofen	10	2.11±0.02	2.13±0.02^{a}	2.19±0.01^{a}	2.27±0.01^{a}	2.21±0.02^{a}	2.19±0.01^{a}	2.15±0.01^{a}	2.26±0.01^{a}	2.17±0.01^{a}	2.19±0.01^{a}	2.15±0.01^{a}
3	RREO	50	2.18±0.03^{ab}	2.29±0.03^{b}	2.29±0.05^{b}	2.44±0.03^{b}	2.39±0.02^{b}	2.40±0.04^{b}	2.35±0.04	2.30±0.02	2.29±0.02	2.23±0.02	2.27±0.01
4		100	2.19±0.04^{ab}	2.31±0.06^{b}	2.40±0.06^{b}	2.47±0.03^{b}	2.45±0.02^{b}	2.36±0.03^{b}	2.30±0.03^{b}	2.28±0.02^{b}	2.32±0.01^{b}	2.24±0.01^{b}	2.24±0.01^{b}

(one way ANOVA followed by dunetts multiple comparison test); ^{a}=significant ($p<0.05$) as compared to control; ^{b}=significant ($p<0.05$) as compared to drug; RREO=R*abdosia rugosus* essential oil

Table 4: Anti-nociceptive activity of essential oils of *Rabdosia rugosus* (Hot Plate Method) (Mean±SE, n=6)

Groups	Treatments	Dose (mg/kg)	Hot plate reaction time (min)					
			0	30	60	90	120	150
1	Control (Saline water)	0.02 ml	3.00±0.04	2.99±0.03	2.97±0.04	2.89±0.03	2.88±0.05	2.87±0.02
2	Indomethacin	05	3.25±0.04[a]	3.84±0.04[a]	4.96±0.05[a]	4.21±0.05[a]	4.06±0.05[a]	3.84±0.04[a]
3	RREO	50	2.97±0.03	3.02±0.03	2.98±0.03	2.89±0.03	2.92±0.02	2.94±0.06
4		100	2.98±0.03	2.95±0.04	2.99±0.03	2.89±0.04	2.93±0.02	2.86±0.02

(one way ANOVA followed by dunetts multiple comparison test); [a]=significant ($p<0.05$) as compared to control; [b]=significant ($p<0.05$) as compared to drug; RREO=*Rabdosia rugosus* essential oil

Table 5: Anti-nociceptive activity of essential oils of *Rabdosia rugosus* (Writhing effect) (Mean±SE, n=6)

Group	Treatments	Dose (mg/kg)	Numbers of writhings	% Writhings	% Inhibition
1	Control	0.20 ml	140.50±1.88	100.00	-
2	Ibuprofen	40	79.33±2.11[a]	56.46	43.54
3	RREO	50	135.33±1.05[b]	96.32	3.68
4		100	133.67±1.17[ab]	95.14	4.86

(one way ANOVA followed by dunetts multiple comparison test); [a]=significant ($p<0.05$) as compared to control; [b]=significant ($p<0.05$) as compared to drug; RREO=*Rabdosia rugosus* essential oil

Table 6: Effect of essential oils of *Rabdosia rugosus* on yeast induced pyrexia in mice (Mean±SE, n=6)

Groups	Treatments	Dose (mg/kg)	Body Temp. before administration of drug (°C)		Body Temp. after administration of drug (°C)			
			-18 hrs	0 hrs	1 hr	2 hrs	3 hrs	6 hrs
1	Control	0.20 ml	37.31±0.02	38.43±0.05	38.64±0.05	38.46±0.12	38.61±0.06	38.40±0.07
2	Paracetamol	33	37.26±0.01	38.42±0.05	37.42±0.08[a] (86.31)	37.39±0.02[a] (88.76)	37.40±0.02[a] (87.90)	37.30±0.02[a] (96.69)
3	RREO	50	37.29±0.01	38.44±0.03	38.33±0.02[ab]	37.87±0.03[ab]	37.71±0.03[ab]	37.68±0.02[ab]
4		100	37.28±0.02	38.51±0.03	38.37±0.03[ab]	37.80±0.03[ab]	37.64±0.03 [ab]	37.62±0.02[ab]

(one way ANOVA followed by dunetts multiple comparison test); [a]=significant ($p<0.05$) as compared to control; [b]=significant ($p<0.05$) as compared to drug; RREO=*Rabdosia rugosus* essential oil

responses in mice and showed inhibition of only 3.68% and 4.86% compared to control. Standard drug, ibuprofen significantly reduced writhing responses (43.54%) induced by acetic acid.

Administration of the yeast to the rats produced significant increase in rectal temperature, 18 h after *Sacchromyces cerevisiae* injection. RREO showed significant activity less than the paracetamol as compared to control. Maximum inhibition of pyrexia was shown by RREO (72.84%) at dose of 100 mg/kg b. wt. at 6 hr. The observations of the antipyretic activity of the essential oil are presented in Table 6.

Acute Toxicity

RREO administered intraperitoneally and orally at doses of 150, 300, 450 and 600 mg/kg b. wt. The Swiss albino mice were observed during the first two hours for poisonous symptoms and then mortality was recorded for each treated group at 24, 48 and 72 h after essential oil administration. RREO did not cause any behavioral changes and no death was observed, thus it was considered to be practically non-toxic components.

Effect of essential oil, agonists and antagonists on duodenal smooth muscle

Essential oil of *R. rugosus* (RREO) (2000 μg and 4000 μg) induced least to mild degree of relaxation in the duodenal tissue. However, relaxation disappeared within 15-30 min of washing with Tyrode solution in oils treated duodenal tissue. The normal response of duodenal smooth muscles to ACh (2 μg) did not alter before and after exposure of the oils, and returned to the base line immediately after maximal contraction. Pre-treatment with atropine sulphate (2 μg) inhibited the ACh induced contraction in duodenal smooth muscles, however the relaxation induced by the oil (RREO) at the concentrations of 2000 μg and 4000 μg produced same effect as produced before the treatment of atropine sulphate. Though ACh induced tissue response is blocked by the muscarinic receptor antagonist atropine sulphate, the same antagonist did not alter the RREO (2000 μg and 4000 μg) induced relaxation and also did not affect the ACh induced contraction of duodenum smooth muscles. In addition, it is suggested that the oil induced relaxation did not involve blocking of acetylcholine acting muscarinic receptors. The primary action of acetylcholine to produce contraction of smooth muscles occurs through muscarinic receptors by causing depolarization of the cell membrane through increasing the Na^+ and Ca^{2+} conductance [25].

Therefore, these observations suggested that the oil did not affect both the muscarinic receptor response and activity of ACh of the duodenal smooth muscles might be due to the unknown mechanism involving Na^+ and Ca^{2+} ion channels mediated depolarization of the cell membrane. Adrenaline (1 and 2 μg) caused relaxation of tissue which returned to

Table 7: Antibacterial activity of the essential oil of *Rabdosia rugosus*

Sl. No.	Samples (400 μg/disc)	Test bacteria {Zone of inhibition (mm)}	
		Staphyllococus aureus	*Salmonella enterica enterica*
1	Control	0.00±0.0	0.00±0.0
2	Ampicilline (30 μg/disc)	33.30±0.2[g]	28.30±0.4[g]
3	RREO	10.87±0.5[c]	10.87±0.5[c]

Values are means of three replications±SE. Means with the same letter are not significantly different at $p \leq 0.05$. RREO=*Rabdosia rugosus* essential oil

Table 8: Antifungal activity of essential oil of *Rabdosia rugosus*

Sample	Conc. (ppm)	Growth Diameter (mm)			% inhibition		
		Fusarium oxysporum	*Rhizoctonia solani*	*Sclerotium rolfsii*	*Fusarium oxysporum*	*Rhizoctonia solani*	*Sclerotium rolfsii*
Control		35.00±0.2	35.00±0.3	35.33±0.6	00.00	00.00	00.00
RREO	100	12.73±0.4*	10.05±0.4*	12.73±0.2*	63.62	71.29	63.96
	250	05.53±0.3*	04.77±0.2*	09.07±0.3*	84.27	86.38	74.34

Values are means of three replications±SE, One Way Analysis of Variance (*Multiple Comparisons versus Control Group (Dunnett's Method): $p < 0.050$. RREO=*Rabdosia rugosus* essential oil

base line after 5 min. Treatment of tissue with propranolol (100 μg) blocked adrenaline (2 μg) induced relaxation but mild relaxation induced by RREO (2000 μg, 4000 μg) was diminished with time. Relaxation of GIT smooth muscles by adrenaline is mediated through α- and β-adrenergic receptors. Propranolol, a non-selective β-blocker, enters to block the site for the adrenaline induced relaxation of GIT smooth muscles i.e. adrenergic receptors. However, propranolol did not block the oil induced duodenal smooth muscle relaxation which suggested that the oil induced relaxation is not mediated through adrenergic receptors [26]. In another study, Bazerra, *et al.*, [27] reported GIT smooth muscle relaxation which was not blocked by adrenergic antagonists.Thus, the effect of oil induced adrenergic relaxation occurs probably due to their inhibitory effect on influx of Ca^{2+} through cell membrane of mice duodenal smooth muscles.

Antibacterial Activity and Antifungal Activity

The essential oil components act on outer membrane permeability in gram-negative bacteria [28]. Zone of inhibition of standard drug (ampicilline), 33.30 ± 0.5 mm against *S. aureus* and 28.30 ± 0.5 mm against *S. enterica enterica.* Essential oil of *R. rugosus* (RREO) showed moderate antibacterial activity against the tested pathogenic bacterial strains. The activity of RREO showed a zone of inhibition, 10.87 ± 0.5 mm against *S. aureus* and 10.87 ± 0.5 mm against *S. enterica enterica* (Table 7).

This investigation also reveals that essential oils obtained from *R. rugosus* exhibit a good antifungal activity in terms of % inhibition produced by the tested sample against three plant pathogen, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* recorded in Table 8. RREO showed maximum % of inhibition of *Fusarium oxysporum* (84.27%), *Rhizoctonia solani* (86.38%) and *Sclerotium rolfsii* (74.34%) at 250 ppm concentration.

In previous observation showed that oxygenated terpenoids (such as alcoholic and phenolic terpenes) have effective antimicrobial activity than the other terpenoids components (monoterpene hydrocarbons) [29]. The essential oil of *R. rugosus* was rich in sesquiterpenoids (~90%) and was poor in monoterpenoids (8.1%). α-bisabolol (41.91%) was the major constituent of the oil and the other identified major compounds were germacrene-D (9.70%), β-caryophyllene (7.56%), dehydroabietane (5.24%), ar-curcumene (5.0), *trans*-ferruginol (3.31%) α-cadinol (3.17%), T-muurolol (2.48%), p-cymene (3.21%) and γ-terpinene (2.00%). Hence the antimicrobial activity of essential oils might be due to the oxygenated terpenoids or synergetic effects of other major and minor constituents of the oils.

CONCLUSION

The study indicates the wide chemical variations in the essential oils of *Rabdosia rugosus* from previously reported by other researchers in both qualitatively and quantitatively. *Rabdosia rugosus* essential oil appear to be good and safe natural antimicrobial agent in the control of various human, animal and plant disease and could also be of significance in antipyretic. Further studies should be done to search for new biological active components from essential oil.

AUTHORS CONTRIBUTIONS

Prakash Singh, A.K. Pant and Mahesh Kumar planned and carried out all the experiments in this study. Ravendra Kumar and Om Prakash helped in writing and paraphrasing the work, Valary A. Isidorov and Lech Szczepaniak also played an important role in proofreading and editing the work.

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