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Phytochemical analysis of root extracts of *Rhynchostylis retusa* (L.) Blume from the Eastern Ghats of India

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ABSTRACT

Rhynchostylis retusa (Orchidaceae) is an important ethnomedicinal herb in Indian systems of medicine. Tribal healers commonly employ the roots of this epiphytic orchid to treat various ailments. In the present study, the shade-dried root powder of *R. retusa* was subjected to cold extraction with four solvents, such as n-hexane, acetone, ethyl acetate, and methanol. The crude root extracts were then taken for qualitative phytochemical screening. Furthermore, GC-MS analysis of ethyl acetate and methanol root extracts was carried out. The methanol root extract of *R. retusa* showed a positive result for all the phytochemicals tested except for saponins. GC-MS analysis of *R. retusa* methanol root extract revealed the presence of 16 phytoconstituents. Major phytoconstituents such as 9-Hexadecenoic acid, methyl ester, [Z] Heptadecanoic acid, 16-methyl-, methyl ester, Ethanone,1-[4-(4-morpholyl benzylidene amino)phenyl]-, are recorded in the methanol root extract. In the present study, both methanol and ethyl acetate root extracts showed the presence of9-Hexadecenoic acid, methyl ester, [Z] as the major phytocompound. The phytocompounds identified in methanol and ethyl acetate root extracts exhibit various biological activities, including anti-inflammatory, antibacterial, antifungal, antioxidant, and anticancer properties. Based on the findings of the current study, it can be inferred that the roots of *R. retusa* contain diverse bioactive compounds with medicinal properties. Further investigation of these *R. retusa* root extracts holds the potential for the discovery and development of innovative pharmaceuticals.

Keywords: *Rhynchostylis retusa* (Orchidaceae), Root, GC-MS, Bioactive compounds

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INTRODUCTION

Rhynchostylis retusa (L.) Blume (Orchidaceae) commonly known as foxtail orchid, is an important ethnomedicinal herb. In India, *R. retusa* is distributed in the North East Himalayas, North West Himalayas, Eastern Ghats, and Western Ghats. Every plant part of this epiphytic orchid has therapeutic properties (Roy *et al.*, 2007; Hossain, 2011). The roots of *R. retusa* are used by tribal healers in the Eastern Ghats to treat rheumatism. The tribals of Darjeeling Himalaya use the roots of this plant to cure blood dysentery and wounds (Rahamtulla *et al.*, 2020). Tribals in Nagaland employ *R. retusa* root paste along with *Pisum sativum* leaf buds to treat blood dysentery (Dash *et al.*, 2008; Nongdam, 2014). The root juice of this plant is administered to cuts and wounds by traditional medical practitioners in Nepal (Subedi *et al.*, 2013). The folk healers of Sikkim use the decoction of the root to treat menstrual pain and arthritis (Panda & Mandal, 2013).

Many pharmacological studies have been carried out on this plant (Bhattacharjee & Islam, 2015; Bhatnagar *et al.*, 2017; Rohani *et al.*, 2018). For the development of natural drugs, it is essential to understand the phytochemical composition of medicinal plants. It provides a scientific basis for developing plant-based remedies and traditional medicine practices. Therefore, in the present study, an attempt has been made to unravel the bioactive compounds of *R. retusa* root extracts.

MATERIAL AND METHODS

Collection of Plant Materials

Plant materials of *Rhynchostylis retusa* (Figure 1) were collected from the forest regions of the Eastern Ghats of Andhra Pradesh (India). The plant was identified by referring to standard literature (Abraham & Vatsala, 1981; Deva & Naithani, 1986; Lucksom, 2007). A voucher specimen (ANUBH 1920) was deposited at the herbarium of the Department of Botany and Microbiology for future reference. In mid-October, mature plants from the forest regions were selected for healthy roots, which were then placed in polythene bags. Afterward, the plant parts (roots) were carefully taken out of the polythene bags,

Figure 1: *Rhynchostylis retusa* (L.) Blume

cleaned under running water, chopped into small pieces, and allowed to dry in the shade for a period of 25 to 30 days.

Preparation of Root Extracts

The shade-dried root material was pulverized using an electric blender. The powdered substance was sieved and placed in airtight glass bottles. About 50 grams of powdered root material was extracted separately in 500 mL of n-hexane, acetone, ethyl acetate, and methanol using the cold extraction method at room temperature with gentle stirring for 48 hours. Finally, the extracts were filtered using Whatman No. 1 filter paper, put into glass jars, and kept at 4 °C.

Qualitative Phytochemical Screening of Crude Extracts

The root extracts of *R. retusa* were used for the qualitative identification of primary (carbohydrates and proteins) and secondary metabolites (alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids). The preliminary phytochemical screening was carried out by the standard protocols developed by Harborne (1973) and Trease and Evans (1989, 1996).

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) is an important tool for analyzing unknown plant components. The root extracts of *R. retusa* were subjected to GC-MS analysis. The root extract was loaded into an HP-5 column (30 m X 0.25 mm with 0.25 m film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. The following chromatographic settings were used when operating the equipment: helium as the carrier gas, a flow rate of 1 mL/min; and the injector was set to 200 °C, with the column oven temperature set to 50-250 °C at a rate of 10 °C/min injection mode. The following Mass Spectroscopy settings were used: ionisation voltage of 70 eV; ion source temperature of 250 °C; interface temperature of 250 °C; and mass range of 50-600 mass units.

Identification of Phytocomponents

The GC-MS mass spectrum was interpreted using the National Institute Standard and Technology (NIST) database, which has over 62,000 patterns. The mass spectrum of the unknown phytocomponents was compared to the spectrum of the known components recorded in the NIST library. The name, molecular mass, and structure of the phytocomponents obtained from the plant extract were then determined.

RESULTS AND DISCUSSION

The *R. retusa* has long been used as a medicinal herb to cure a variety of diseases. In India, ethnic groups and traditional healers employ the roots of this plant to treat a variety of ailments such as arthritis, blood dysentery, cuts, wounds, and menstrual pain. In general, chemical components found in the plant contribute to its therapeutic efficacy (Duraipandiyan *et al.*, 2006).

Preliminary Phytochemical Analysis

Phytochemical analysis of four solvent extracts of *R. retusa* roots revealed the presence of different phytochemicals (Table 1). Methanol and ethyl acetate root extracts have been found to contain a greater number of phytochemicals. Methanol root extract showed a positive result for eight phytochemicals except saponins (Table 1). Only the presence of tannins was evident in the acetone root extract. The n-hexane root extract showed no phytochemicals. Differences in the presence of phytochemical substances in root extracts may be related to the compound's solubility in the solvent used (Bako *et al.*, 2005). The results of this study are consistent with those of several previous investigations that have also identified alkaloids, carbohydrates, coumarins, flavonoids, phenols, steroids, tannins, and terpenoids in a variety of orchid species, including *Acampe praemorsa* (Suja & Williams, 2016), *Aerides odorata* (Katta *et al.*, 2019), *Cymbidium aloifolium* (Shubha & Chowdappa, 2016), *Luisia zeylanica* (Sohag *et al.*, 2017) and *Vanda tessellata* (Biswas & Sinha, 2020).

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of *R. retusa* root extracts

GC-MS analysis of *R. retusa* methanol root extract revealed the presence of 16 phytoconstituents in its gas chromatogram (Figure 2). Structures of 16 phytoconstituents along with their retention time, molar mass, and peak area % are displayed in Table 2. Major phytoconstituents such as 9-Hexadecenoic acid, methyl ester, [Z] (Figure 3), Heptadecanoic acid, 16-methyl-, methyl ester, Ethanone,1-[4-(4-morpholyl benzylidene amino) phenyl]-, 8-Octadecenoic acid, methyl ester, Dodecanoic acid, 11-oxo-, methyl ester are observed in the methanol root extract (Table 2).

GC-MS chromatogram of ethyl acetate root extract showed 14 peaks which indicated the presence of 14 different phytochemical compounds (Figure 4). Major phytoconstituents

Table 1: Phytochemical profile of *Rhynchostylis retusa* root extracts

S. No.	Phytochemicals	Test/Reagents used	n-hexane	acetone	ethyl acetate	methanol
1	Carbohydrates	Benedict's test	-	-	+	+
2	Proteins	Biuret test	-	-	-	+
3	Alkaloids	Dragendorff's test	-	-	+	+
4	Flavonoids	1% $AlCl_3$ test	-	-	+	+
5	Phenols	10% $FeCl_3$ Test	-	-	+	+
6	Saponins	Frothing Test	-	-	-	-
7	Steroids	Liebermann-Burchard Test	-	-	-	+
8	Tannins	Iodine Test	-	+	+	+
9	Terpenoids	Salkowski Test	-	-	+	+

Table 2: Phytocompounds recorded in the methanol root extract of *R. retusa*

S. No.	Compound	RT	Mass	Peak Area %
1	Pteridine, 2-methyl-	12.23	146.0000	2.932
2	Butyric acid, 4-Butoxy-, methyl ester	12.73	174.0000	3.367
3	Nonanoic acid, 1-methyl ethyl ester	13.5	200.0000	3.214
4	Isosativene	15.07	204.0000	3.233
5	Pentadecane	15.77	212.0000	4.621
6	Flavone	16.37	222.0000	5.329
7	Dodecanoic acid, 11-oxo-, methyl ester	16.85	228.0000	7.814
8	9-Hexadecenoic acid, methyl ester, [Z]	17	268.0000	10.412
9	Hexadecanoic acid, methyl ester	17.25	270.0000	10.018
10	9-Eicosene, [E]-	17.88	280.0000	5.253
11	8-Octadecenoic acid, methyl ester	18.98	296.0000	10.120
12	Heptadecanoic acid, 16-methyl-, methyl ester	19.2	298.0000	10.373
13	Ethanone, 1-[4-(4-morpho lylbenzylidenamino) phenyl]-	19.78	308.0000	10.334
14	Isopropyl Linoleate	21.62	322.0000	4.681
15	Elaidic acid, Isopropyl ester	23.4	324.0000	3.804
16	1- Tetradecene, 2-decyl-	26.08	336.0000	4.488

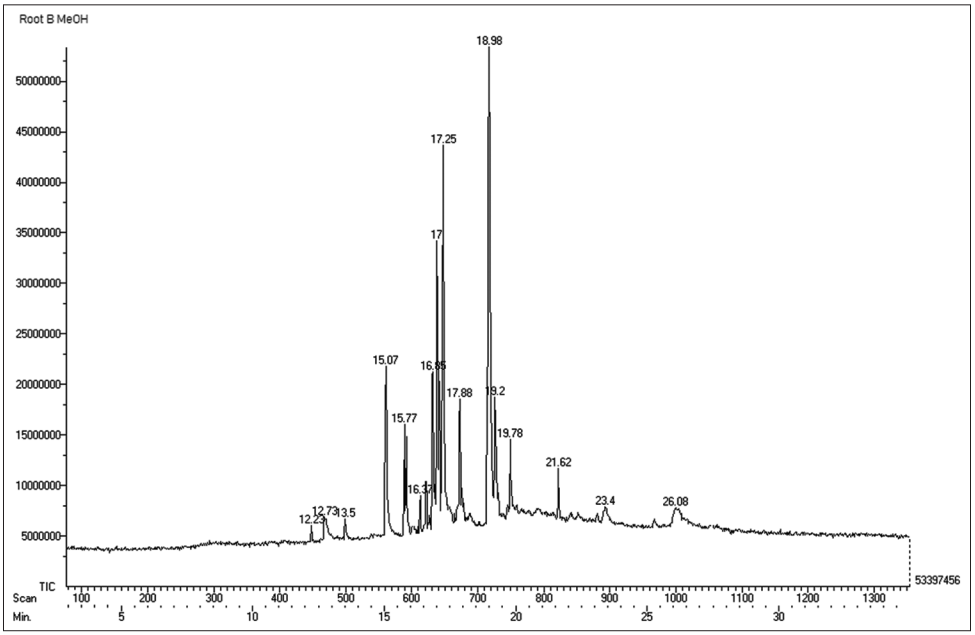

Figure 2: GC-MS chromatogram of methanol root extract of *R. retusa*

such as 9-Hexadecanoic acid, methyl ester, [Z]-,Hexadecanoic acid, methyl ester, 8-Octadecanoic acid, methyl ester, Z-6-Tetradecen-1-ol acetate, 1-Tricosanol, Flavone, 9-Tricosene, [Z]-, are identified in the ethyl acetate root extract of *R. retusa* (Table 3).

In the present study, both methanol and ethyl acetate root extracts showed the presence of 9-Hexadecenoic acid, methyl ester, [Z] as the major phytocompound. This compound possesses significant antioxidant activities (Rahman *et al.*, 2014). The methanol root extract of *R. retusa* contains a significant

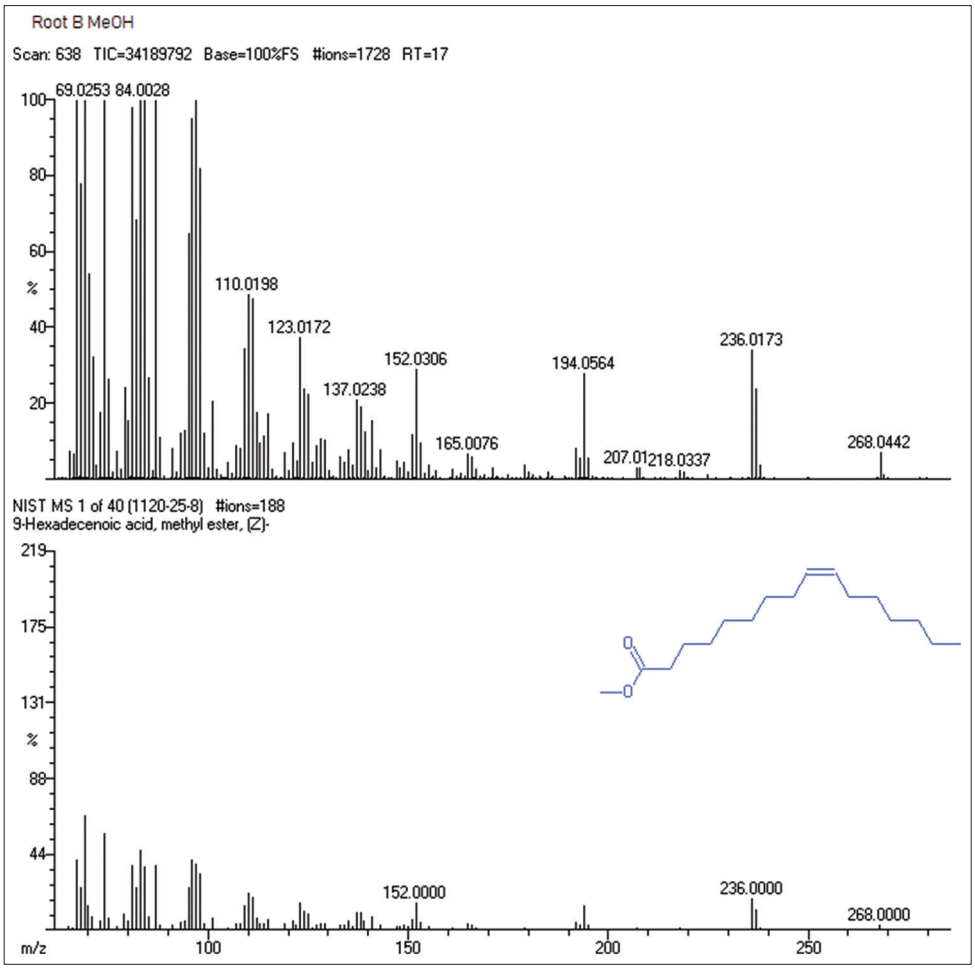

Figure 3: Major phytocompound 9-Hexadecenoic acid, methyl ester, [Z] recorded in the methanol root extract of *R. retusa*

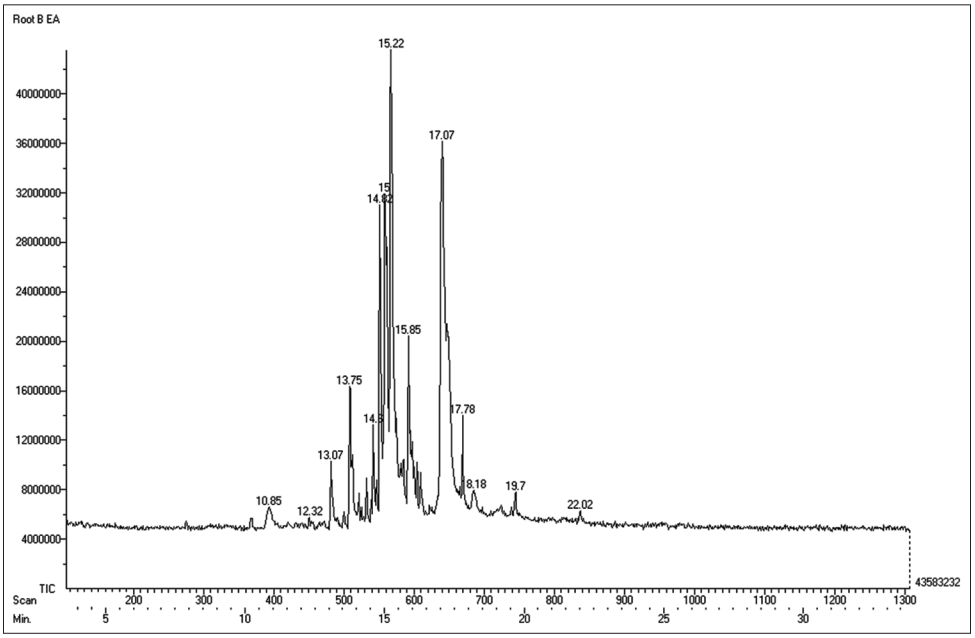

Figure 4: GC-MS chromatogram of ethyl acetate root extract of *R. retusa*

Table 3: Phytocompounds recorded in ethyl acetate root extract of *R. retusa*

S. No.	Compound	RT	Mass	Peak area %
1	4-Hydroxy, 3-Methoxy benzyl alcohol	10.85	154.0000	3.840
2	1,5-Napthalenediol, decahydro-	12.35	170.0000	3.592
3	Humulen-[v1]	13.07	204.0000	4.105
4	Phenol, 2,4-bis[1,1-dimethylethyl]-	13.75	206.0000	5.013
5	Flavone	14.6	222.0000	6.625
6	Z-6-Tetradecen-1-ol acetate	14.82	254.0000	10.507
7	9-Hexadecanoic acid, methyl ester,[Z]-	15	268.0000	14.438
8	Hexadecanoic acid, methyl ester	15.22	270.0000	14.124
9	1-Tricosanol	15.85	280.0000	7.057
10	8-Octadecanoic acid, methyl ester	17.07	264.0000	12.339
11	9-Tricosene, [Z]-	17.78	322.0000	5.454
12	2,6,10,14-Hexadecatetraenoicacid, 3,7,11,15-tetramethyl-, ethyl ester, all E-	18.18	332.0000	4.757
13	1-Tetradecene, 2-Decyl-	19.7	336.0000	4.272
14	Methoxy acetic acid, octadecyl ester	22.02	342.0000	3.870

number of fatty acid methyl ester compounds, which include Butyric acid, 4-Butoxy-, methyl ester, Dodecanoic acid, 11-oxo-, methyl ester, Hexadecanoic acid, methyl ester, 8-Octadecenoic acid, methyl ester, Heptadecanoic acid, 16-methyl-, methyl ester (Table 2). In both the methanol and ethyl acetate root extracts, hexadecanoic acid, methyl ester, is detected. Pinto *et al.* (2017) reported that the above-mentioned phytocompound possesses both antioxidant and antifungal properties. In both of the *R. retusa* root extracts, the phytocompound such as 8-octadecanoic acid methyl ester is also detected. According to Shahin *et al.* (2006), this compound affects the most important serum lipid in lactating women.

The methanol root extract of *R. retusa* has been found to contain heptadecanoic acid, 16-methyl-, methyl ester. Kandasamy *et al.* (2012) previously identified this compound as the most abundant in crude methanol extract of the fungus *Trichoderma*. Furthermore, their research studies revealed that this fatty acid derivative is a potent inhibitor of the skin cancer protein (4,5-Diarylisoxazole HSP90 Chaperone).

The phytocompound 4-hydroxy-3-methoxybenzyl alcohol, commonly known as Vanillyl alcohol, has been identified in ethyl acetate root extract of *R. retusa* and has been employed as an anti-inflammatory, antiangiogenic, and anti-nociceptive agent (Jung *et al.*, 2008).

Phenol,2,4-bis[1,1-dimethylethyl]- is recorded in the ethyl acetate root extract of *R. retusa*. Phenols and their derivatives are efficient radical scavengers, according to several studies that looked into the antioxidant properties of phenolic compounds (Velioglu *et al.*, 1998; Li *et al.*, 2007). Phenolic compounds have been linked to a lower risk of liver, lung, and cervical cancers (Araujo *et al.*, 2011; Lee *et al.*, 2012; Stagos *et al.*, 2012). Mahbub *et al.* (2013) suggest that these compounds may be therapeutic agents for the treatment of leukemia by causing decreased cell viability and inducing apoptosis. According to Anantharaju *et al.* (2016), they control cell division, survival, and apoptosis.

Flavone is recorded in both methanol and ethyl acetate root extracts of *R. retusa*. Flavones are benzopyran natural compounds that form a significant category of oxygen heterocycles that are found extensively in the plant kingdom as secondary metabolites. These compounds possess anti-inflammatory, anti-oestrogenic, antimicrobial, anti-allergic, antioxidant, and antitumor activity (Cushnie & Lamb, 2005; Verma & Pratap, 2010).

1-Tricosanol has been recorded in the ethyl acetate root extract of *R. retusa*. This fatty alcohol poses antimicrobial and anti-inflammatory properties (Anjali *et al.*, 2019).

Both the methanol and ethyl acetate root extracts contain the phytocompound known as 1-Tetradecene, 2-Decyl-. Earlier this compound was also reported in ethyl acetate leaf extract of *Dendrobium aphyllum* (Rahamtulla *et al.*, 2023). According to the studies of Fernandes & Krishnan (2019) and Taduri *et al.* (2022) this compound possesses anti-inflammatory, anti-arthritic, and antioxidant properties.

The information above supports the use of roots by native healers to treat an extensive variety of ailments and proves the therapeutic efficacy of *R. retusa*. Additionally, this plant includes several important bioactive compounds that have been shown to possess anti-inflammatory, antibacterial, antifungal, antioxidant, and anticancer properties.

CONCLUSION

The findings of the present investigation revealed that the methanol and ethyl acetate root extracts of *R. retusa* contain numerous bioactive compounds such as 9-Hexadecenoic acid, methyl ester, [Z], flavone, hexadecanoic acid, methyl ester, 1-Tricosanol and 1-Tetradecene, 2-Decyl-. These phytocompounds possess significant therapeutic properties that can be employed in treating a variety of ailments. Further research on this ethnomedicinal plant may aid in the isolation of therapeutically beneficial chemical compounds. These phytocompounds can then be subjected to pharmacological and clinical studies, facilitating the plant's utilization as an herbal remedy for therapeutic purposes.

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