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Research Article

Phytochemical and FT-IR Spectral Analysis of *Rhododendron arboreum* Sm. ssp. *nilagiricum* (Zenker) Tagg.

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Abstract

Rhododendron arboreum Sm. ssp. *nilagiricum* is an endemic medicinal plant in Southern Western Ghats of India. The present investigation was focused on the preliminary phytochemical screening and Fourier Transform Infrared Spectral analysis of *R. arboretum* ssp. *nilagiricum*. The aqueous and various organic solvent extracts of acetone, Benzene, Chloroform, Ethanol and Petroleum ether from the leaves of *R. arboretum* ssp. *nilagiricum* were tested for the presence of phytochemical constituents. FT-IR analysis was carried out to characterize the bioactive constituents present in the leaves powder of *R. arboreum* ssp. *nilagiricum*. The qualitative analysis of phytoconstituents such as phenols, flavonoids, quinone, steroids, tannins, xanthoprotein and coumarins were richly present in both acetone and ethanol extracts compared to other extracts. The FT-IR spectrum showed the presence of Carbonyls (C=O), Phenol (C-O), Thioethers(C-S), Disulphids (S-S), Normal polymeric O-H, phenolic compounds and aryl thio ethers. The present investigation can be used for further research into the finding of novel potent phytodrugs from different medicinal plants.

INTRODUCTION

Medicinal plants have recently received the attention of the pharmaceutical and scientific communities and various publications have documented the therapeutic value of natural compounds in a bid to validate claims of their biological activity (Ncube *et al*., 2008). Plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al*., 1999). The different phytocontituents present in medicinal plants such as flavonoid, alkaloid, phenol and tannins, carboxylic acids, terpenes and aminoacids and inorganic acids (Florence *et al*., 2014). These phytoconstituents present specific distinctiveness and properties and it has shown protective effects against diseases without reducing their therapeutic efficacy and contains a wide range of bioactive compounds that can be used to treat various infectious diseases (De Britto *et al*., 2012).

Phytochemical characterization of medicinal plants always leads a major role for better determining of drug in various aspects (Yadav and Dixit, 2008; Dhale and Mogle, 2011; Murugan and Mohan, 2011; Devi *et al*., 2012). A variety of techniques can be used to characterize and determine the presences of such phytocontituents in medicinal plants. Spectroscopic techniques are the most useful and popular tools used for this purpose. The Fourier Transform Infrared (FT-IR) spectroscopy allows the analysis of a relevant amount of compositional and structural information in plants.

Moreover, FT-IR spectroscopy is an established time saving method to characterize and identified functional groups (Grube *et al*., 2008).

Rhododendron arboreum Sm. ssp. *Nilagiricum* (Zenker) Tagg. (Family; Ericaceae) is an endemic medicinal species found in Southern western Ghats. The leaves were reported to contain flavonoids (Kamil and Shafiullah, 1995) and phenolic compounds (Sharma *et al*., 2010) were found to have potent antioxidant (Prakash *et al*., 2007) anti-inflammatory (Sharma *et al*., 2009) and hepatoprotective activity (Prakash *et al*., 2008). In view of this fact (Gautam *et al*., 2016; Liu *et al*., 2016; Tuan *et al*., 2017) the present investigation was carried out to screen the phytoconstituents present in aqueous and organic solvent extracts from the leaves of *R. arboreum* ssp. *nilagiricum* and to determine their functional group using (FT-IR) spectral analysis.

MATERIALS AND METHODS

Plant material

Mature leaves of *Rhododendron arboreum* ssp. *nilagiricum* were collected from the Palni Hills of Southern Western Ghats, India. The leaves were washed with sterile water, shade dried, chopped into small pieces and ground coarsely to powder using a mixer grinder. 25 g of the dried plant material was used for extraction.

Extraction

25 gms of the powdered material continuously extracted with 100 ml of acetone, Benzene, Chloroform, Ethanol, Petroleum ether and Aqueous for 18 hrs at 60°C using Soxhlet apparatus. The extract was filtered through Whattman No.1 filter paper. The filtered extract was evaporated by a rotary vaccum evaporator. The evaporated residue with constant weight was stored prior to analyses in dark at 4°C.

Qualitative phytochemical analysis

The extracts were tested for the presence of bioactive compounds by using following standard methods (Harborne, 1973).

Fourier Transform Infrared Spectroscopic Analysis (FT-IR)

The leaf sample was oven dried at 60°C and ground into fine powder through agate mortar. Two milligrams of the sample were mixed with 100 mg KBr (FT-IR grade) and then compressed to prepare salt-disc (3mm diameter). The disc was immediately put into the sample holder and FT-IR spectra was recorded in the absorption range between 400 and 4000 cm^{-1}. All investigations were carried with a SHIMADZU FT-IR spectrometer.

RESULTS AND DISCUSSION

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The phytochemical screening of the different extracts of *R. arboreum* ssp. *nilagiricum* showed the presence of secondary metabolites including phenols, flavonoids, saponins, quinones, steroids, tannins, xanthoproteins, coumarins and carbohydrates which has great medicinal properties, whereas it showed the absence of some important bioactive compounds of alkaloids and carboxylic acid (Table 1).

The very strong absorption bands at 3379.05 cm^{-1} are the representative for normal polymeric O-H stretching vibrations. The 1612.38 cm^{-1} band is due to the stretching vibration of C=O stretch carbonyls, characteristic of the secondary amides and other compounds. The bands 1400.22 and 1200 cm^{-1} are due to the bending vibration of phenol C–O stretch, characteristic of the presence of phenolic compounds. The strong band occurs at 1124.42 cm^{-1} in the sample predicts the presence of C–O stretching alcohol. The secondary peaks at 752.19 cm^{-1} in the spectrum also indicate the presence of aryl thio ethers, ϕ– S (C–S stretch). The absorbance bands at 644.18 and 603.68 cm^{-1} represents the thioethers, CH_3–S– (C–S stretch) and disulphides (S–S stretch) (Figure 1). Phytochemical analysis of the plant extracts revealed the presence of phytoconstituents which are known to exhibit medicinal properties (Sofowra, 1993). Maximum number phytochemicals present in the acetone and ethanol leaf extracts of *Rhododendron arbortum* ssp. *nilagiricum*.

The phenolic compounds are one of the largest and most important groups of plant metabolites (Singh *et al*., 2007). Flavonoids are effective antioxidant and show strong anticancer activities (Okwu, 2004). The leaves of *R. arboreum* ssp. *Nilagiricum* revealed that the presence of 3-D – galactoside of quercetin is a triterpenoid compound leads to anticancer activities (Ranganathan and Sambamurthy, 1959).The plant extracts were also revealed to contain saponins, Tannins and steroidswhich are known to produce inhibitory effect on inflammation, ulcerated tissues (Just *et al*., 1998). Steroids have been reported to have antibacterial properties (Raquel, 2007).

Table 1: Phytochemical screening of different extracts of *R. arboreum* ssp. *Nilagiricum*

Phytochemical constituents	Acetone	Benzene	Chloroform	Ethanol	Petroleum ether	H_20
Alkaloids	-	-	-	-	-	-
Phenol	+++	++	+	+++	+	++
Flavonoids	+++	+	+	++	+	+
Saponins	-	+++	+++	-	+++	-
Protein	-	-	+	+	-	-
Quinone	++	-	-	++	-	-
Steroids	++	+	-	+++	+	+
Tannin	+++	-	-	+++	-	+++
Xanthoprotein	+++	-	-	-	-	-
Carboxylic acid	-	-	-	-	-	-
Coumarins	+++	-	+	-	+	-
Carbohydrates	-	+	+++	+	+	-

(+) Low, (++) Average, (+++) High, (-) Absent

The FT-IR spectral analysis signals for the leaf powder *Rhododendron arboreum* ssp. *nilagiricum* have been taken. In this wave number (cm^{-1}) is plotted against percentage transmittance. The FT-IR spectrum exhibits the characteristic finger print band features. The FT-IR spectral signal of the plant powder is represented in the table 2.

Table 2: FT-IR analysis of the leaf powder of *R. arboreum* ssp. *Nilagiricum*

S. No.	Wave number (Cm^{-1})	Type of vibration
1	3379.05	Normal polymeric O–H stretch
2	1612.38	C=O stretch carbonyls
3	1400.22	Phenolic compounds
4	1200	Phenol C–O stretch
5	1124.42	C–O stretching alcohol
6	752.19	Aryl thio ethers, ϕ– S (C–S stretch)
7	644.18	Thioethers, CH_3–S– (C–S stretch)
8	603.68	disulphides (S–S stretch)

FT-IR spectrum can be used to confirm the functional constituent's present in the medicinal plant materials and also to evaluate the qualities of phytoconstituents (Surewicz *et al*., 1993).The results of the present study FT-IR spectrum revealed the functional constituents present in the crude powder of *Rhododendron arboreum* ssp. *nilagiricum*. The FT-IR spectral analysis of *Rhododendron arboreum* ssp. *nilagiricum* spectrum reveals the presence of 8 peaks, which functional groups are present in large quantity. Many researchers applied the FT-IR spectrum as a tool for distinguishing the medicinal plants based on their chemical constituents (Asha *et al*., 2014; Florence and Jeeva, 2015; Florence *et al*., 2015; Lincy *et al*., 2015; Joselin and Jeeva, 2016).

CONCLUSION

Phytochemical screening clearly reveals that the maximum classes of phytoconstituents from the leaves extracts of *Rhododendron arboreum* ssp. *nilagiricum*. Therefore, in order to achieve spectroscopic studies are required for the structural elucidation and identification of active principles present in the leaves of *Rhododendron arboreum* ssp. *nilagiricum*. The identification of phytochemical constituents of this plants having highest therapeutic efficacy and could be develop more novel drugs for various ailments.

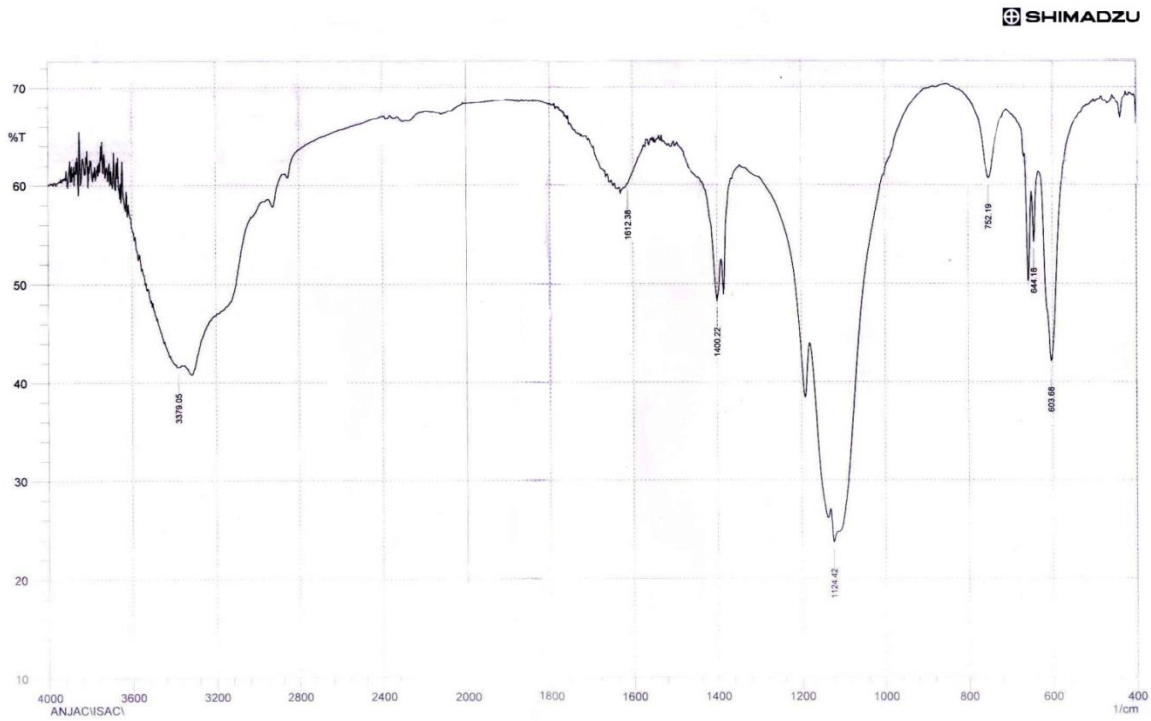

Fig. 1: Fourier transformed spectral analysis of *R. arboreum* ssp. *nilagiricum*

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