Isolation of bioactive compounds of *Taxus baccata* and *Swertia chirata* plants of Uttarakhand region by GC- MS

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***Abstract*: Present study was focused on evaluating bioactive compounds of *Taxus baccata* and *Swertia chirata* using GC-MS. The bioactive components of methanol extract of fruit of *Taxus baccata and* leaves of *Swertia chirata* were investigated by Perkin- Elmer Gas chromatography-mass spectrometry. GC-MS analysis of methanol extract of *Swertia chirata* leaves and *Taxus baccata* fruit revealed the presence of twenty three and nineteen compounds respectively. It can be concluded that *T. baccata* and *S. chirata* plant parts have higher concentration of various terpenoids, tri-terpenoids, and fatty acids compounds and their derivatives that may be responsible for its promising antibacterial activities.**

***Keywords*: GC-MS**

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Introduction

Swertia chirata is a medicinal herbs of traditional of Indian system of Medicine. In Vedas and Samhitas. Among the different species of *Swertia* reported in India, *Swertia chirata* is considered the most important for its medicinal properties. The bitterness, antihelmintic, hypoglycemic and antipyretic properties are attributed to various constituents like amarogentin, swerchirin, swertiamarin and other active principles of the herb. This plant indigenous to temperate Himalaya, consist of 180 species having various medicinal properties and belongs to family Gentianaceae, The trade name of *Swertia chirata* is chiretta. It is found at an altitude of 1200-3000 m (Pradhan and Badola 2010). This plant is effective against gastrointestinal infection (Mukherji 1953), used as antipyretic, hypoglycemic (Bhargava *et al*. 2009), antifungal, (Rehman *et al*. 2001), hepatoprotective, anti-inflammatory (Banerjee *et al.* 2000), antibacterial (Joshi and Dhawan 2005), antioxidative (Scartezzini and Speroni 2000) and used to treat malaria and diabetes (Kumar and Staden 2016).

Taxus baccata (English yew) belongs to family "taxaceae" is an evergreen tree. This plant has been used in the Ayurvedic system for the treatment of cancer, diarrhea and asthma. *T. baccata* leaves are reported to be used in traditional medicine as abortificient, antimalarial, antirheumatic and for bronchitis and dried leaves are used against asthma, Anticancer, anti- inflammatory and antinociceptive, antifungal, antimycobacterial, activity of *T. baccata* has been reported. (Brown 1932, Sing 1995, Jennewein *et al.* 2001, Erdemoglu *et al.* 2001). As both plant are richest source of various medicinal properties, present study was
Focused to identify various phytoconstituents by GC-MS method.

Experimental

Collection of Plant Material

Fresh Plant material were collected from Uttarakhand region, India (Institute of Biotechnology, G. B. Pant University of Agriculture and Technology. After washing under running tap water, plant material (leaves and fruits) were air dried at room temperature for about 15 days. The dried plant material were powdered by using electrical blenders and stored in air tight container in the refrigerator at 4ºC for further analysis.

Preparation of Extract

Dried powder (50 gm) of *Taxus baccata* fruit and *Swertia chirata* leaves were placed inside two different porous cellulose thimbles and placed in an extraction chamber. Extraction was done by using 750 ml methanol filled in the collection flask up to 72 hours. Extracts were concentrated and evaporated to complete dryness using a rotary evaporator under reduced pressure at 40ºC. The dried extracts were stored at -4ºC for further studies.

Gas chromatography –Mass Spectrometry (GC-MS)

HP 6890 gas chromatograph equipped with a FID detector and a HP-5 fused silica column (30 m x 032 mm x 0.21 film thickness), using nitrogen as a carrier gas was used for GC-MS analysis of the *T. baccata and S. chirata* plant parts. The temperature of injector and detector were preserved at 210 and 230^{0}C respectively following the column oven temperature from 60 to 220^{0}C with an increase in rate of 3^{0}C/min.

Mass spectrometry was performed on a Perkin Elmer mass spectrometer (Model Claurus 500) linked to a Perkin Elmer Claurus 500 gas chromatograph with a 60 m x 0.32 mm x 0.2 µm film thickness fused silica column (Rtx-5). Carrier gas was helium at flow rate 1.0 ml/min and the mass range was scanned from 40 to 600 Daltons. Temperature programme range of oven was 60 220^{0}C having an increase in rate of 3^{0}C/mm. The bioactive phytocompounds of the plants methanolic extract were identified on the basis of GC retention indices and by comparing their 70 eV mass spectra with spectrum of known components stored in the NIST & WILEY libraries or reported in literature (Adams 1995, Adams 2007).

Identification of phytocompounds

The identification and characterization of chemical compounds in methanolic extracts was based on GC retention time. Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. Various compounds were identified by their retention time and based on NIST library. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the compounds of the test materials were ascertained.

Result and discussion

The studies on the active principles in the *Swertia chirata* leaf and *Taxus baccata* fruit methanol extract done by GC-MS analysis indicated the presence of 23 compounds and 19 compounds respectively (Table 1). Identified active compounds with their Retention index and concentration (peak area %) are presented in Table 1. tri-terpenoids, terpenoids, and fatty acids such as AR-curcumene, Linalyl acetate, Cymene, Umbellulone, Limonene, α- Pinene, Sabinene and Caryophyllene oxide were the major phytochemicals identified in *Swertia chirata.* The antibacterial activity of terpenoids is known out of these phytochemicals (Jing *et al.* 2013, Das *et al*. 2006).

Terpenoids components that were detected in *Taxus baccata* are α- Pinene, δ-3 carene, Sabinene, Cymene, Limonene, Umbellulone, cis –piperitol, Alpha-Terpinenyl acetate, Caryophyllene oxide *etc*. These valuable phytocomponents are potential bioresources for phytopharmaceutics. Through analyzing the characteristics in *Taxus baccata* and *Swertia chirata*, the researchers showed that there were several Terpenoids in methanol extract. The antimicrobial, anti-inflammatory, chemo-protective, antioxidant and hepatoprotective activities are well known in Umbellulone, Sabinene, Limonene and α- Pinene.

The result of GC-MS conclude that both the plants are richest source of various bioactive phytoconstituents that may be responsible for its antibacterial activities.

Table 1: Phytochemical compounds identified in leaves of *Swertia chirata* and fruits of *Taxus baccata* through GC-MS

S.No.	Components	$RI^{Lit.}$	RI^{Exp}	Peak area % of A	Peak area % of B	Mode of Identification
1	Tricyclene	927	925	tr	tr	RI,MS- Co- inj
2	α-Thujene	929	927	1.6	1.09	RI,MS
3	α- Pinene	935	932	8.0	7.14	RI,MS, Co- inj
4	α- Phellandrene	1008	1006	0.56	0.14	RI,MS, Co- inj
5	Sabinene	1065	1065	7.01	6.64	RI,MS
6	β- Pinene	981	975	0.43	0.46	RI,MS
7	Mycenae	991	989	3.12	1.98	RI,MS Co- inj
8	δ-3 carene	1013	1010	2.23	5.02	RI,MS
9	α- Terpinene	1019	1015	tr	0.16	RI,MS
10	Cymene	1020	1018	3.20	5.97	RI,MS
11	Limonene	1024	1020	16.86	13.96	RI,MS, Co- inj
12	1,8. Cineole	1034	1030	-	-	RI,MS
13	γ– terpinene	1050	1042	3.01	0.46	RI,MS, Co- inj
14	α-terpinolene	1084	1078	1.96	-	RI,MS, Co- inj
15	Linalool	1094	1090	1.62	-	RI,MS, Co- inj
16	Umbellulone	1170	1176	26.85	32.65	RI,MS, Co- inj
17	4-Terpineol	1177	1178	5.06	1.44	RI,MS, Co- inj
18	α- Terpineol	1184	1182	-	-	RI,MS, Co- inj
19	cis -piperitol	1201	1199	-	2.16	RI,MS, Co- inj

20	Bornyl acetate	1278	1270			RI,MS, Co- inj
21	β-citronellol	1224	1220	-	-	RI,MS, Co- inj
22	Linalyl acetate	1335	1330	3.42	-	RI,MS, Co- inj
23	Alpha-Terpinenyl acetate	1298	1210	2.92	5.8	RI,MS
24	α- cubebene	1350	1310	-	tr	RI,MS, Co- inj
25	Caryophyllene oxide	1589	1582	6.0	12.0	RI,MS, Co- inj
26	β- cubebene	1388	1386	0.89	tr	RI,MS, Co- inj
27	AR-curcumene	1481	1478	2.5	-	RI,MS,
28	Sclarene	1985	1983	0.34	-	RI,MS
29	Kaurene	2040	2035	1.8	-	RI,MS
	Total			98.25	97.07	RI,MS

Aberrations used: A= Leaves of *Swertia chirata* B= Fruits of *Taxus baccata*

RI: Retention index on the HP-5 column; $RI^{Lit.}$: retention indices literature (Adams, 2009); $RI^{Exp.}$: retention indices (experiment); –: absent

Conclusion and recommendation

Data obtained through GC-MS analysis showed that *T. baccata* and *S. chirata* plant parts have higher concentration of various terpenoids, tri-terpenoids and fatty acids compounds and their derivatives that may be responsible for its promising medicinal and antibacterial activities. Finally, recommendations can be made for further investigation to be done for different plant parts fractionates for their antibacterial and various medicinal properties. The plant parts extraction should be done with other solvent types also in order to evaluate enough quality and quantity of bioactive compounds.

Conflict of interest

There is no conflict of interest.

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