Chemical Fingerprints of an Indian Traditional Herbal Drug *Talisapatra* (*Abies webbiana*) and Comparison with English yew (*Taxus baccata*)

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ABSTRACT

Present work was carried out with a view to develop chemical fingerprints to differentiate the herbal drugs *Taxus baccata* L. and *Abies webbiana* (Wall ex D. Don) Lindl. Both the plants are commonly called as *Talisapatra* in India and having different medicinal applications. *T. baccata* is native to Europe and *A. webbiana* is found in India and also used in traditional medicine. Dried leaves of both the plant are morphologically similar and in powdered form it is very difficult to differentiate them and hence there are chances for adulteration and also misuse of the herbs. In this project phytochemical, spectroscopic (UV-Visible, FT-IR and NMR) and chromatographic techniques (HPTLC and HPLC) were applied to obtain the chemical fingerprints of selected herbs. *A. webbiana* contained higher level of total phenolic compounds (6301.27 mg GAE / 100 g) when compared to *T. baccata* (977.45 mg GAE / 100 g). UV-Visible absorbance at 577 and 663 nm are unique for *T. baccata*. FT-IR peaks at 3403, 1030 and 577 cm^{-1} were unique for *T. baccata* while *A. webbiana* exhibited unique peak at 3371, 1059 and 613 cm^{-1}. NMR signals revealed remarkable difference between chloroform extracts of *T. baccata* from *A. webbiana*. HPTLC profile exhibited unique bands with Rf value of 0.11, 0.25, 0.62, 0.68, 0.91 and 097 for *T. baccata* and *A. webbiana* exhibited unique spots with Rf value of 0.05, 0.27, 0.38, 0.44, 0.65, 0.72 and 0.93. Unique HPLC peaks for *T. baccata* were 2.07, 2.28, 4.86, 5.08, 5.97 and 6.86 min whereas *A. webbiana* revealed unique peaks at 1.90, 2.00 & 4.52 min. Chemical fingerprint results obtained from the present work would be useful in differentiating *T. baccata* from *A. webbiana*.

Keywords: *Talisapatra*; *Taxus baccata*; *Abies webbiana*; Spectroscopy; Chromatography; Chemical fingerprints.

INTRODUCTION

Cancer is one of the leading health problems in the world and in modern medicine side effects of chemotherapy and radiation therapy have been well known[1]. When looking for alternative potential anti-cancer drugs, certain plant-derived compounds like vincristine, vinbalstin, taxol, etc., yielded positive results. In fact, plants have a long history in the treatment of cancer and in this context *Taxus baccata* L. has proven to be useful in cancer treatment.

T. baccata or English yew (Family: Taxaceae) is distributed throughout the temperate zones of the northern hemisphere[2]. It is a small-to medium-sized evergreen non-resinous gymnosperm tree grows up to 20 m height that historically has been used as medicine[3]. It is commonly called as *Talisapatra* in India[4]. The anticancer properties of taxol were discovered in *T. brevifolia* extracts[5] while Schiff et al.[6] found that the cellular target of taxol was tubulin. Broncho-constriction[7], anti-inflammatory & antinociceptive[8], antimicrobial[9] and anti-cancer activity[10] of *T. baccata* was proved scientifically. *T. baccata* has received considerable interest due to its diterpene alkaloids content, particularly the taxols (Paclitaxol)[11]. Additionally,

lignans[12], lignins[13], baccatin[14], flavonoids, steroids, bicyclic & tetracyclic diterpenes[15], biflavones and taxoids[16] and sugar derivatives have been reported in different parts of this plant. Chemical methods like LC-MS[17], Infrared and Raman spectroscopic techniques[18] have been attempted to detect phytochemicals of *T. baccata*.

In India, *Abies webbiana* (Wall ex D. Don) Lindl is commonly known as *Talispatra* in Hindi and Indian Silver Fir in English. It is a large, tall, evergreen tree occurring in the Himalayan region from Kashmir to Assam states in India[19]. It comes under the Family Pinaceae and leaves of this plant have different uses in Ayurveda for treating chronic obstructive pulmonary diseases, cough, tumor, hypochlorhydria, amoebiasis, iccup, vomiting, helminthiasis and mouth disorders[20, 21]. *A. webbiana* leaf was reported to possess anti-microbial[22], anti-tussive[23], anti-spasmodic, bronchodilator & anti-platelet[24], anti-inflammatory[25] and antioxidant properties[26]. Phytochemical analysis revealed the presence of an alkaloid (1,4'-methoxyphenyl-aziridine)[27] and biflavonoid (Abiesin)[28] in this plant.

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Since both *T. baccata* and *A. webbiana* are called commonly as *Talisapatra*, there is confusion in their usage and also possibilities for adulteration due to their similar morphology. Even though the medicinal properties and chemical constituents of selected plants are different, their common name and morphology causes confusion in their identity. Hence, we have carried out the present work to differentiate the *T. baccata* from *A. webbiana* using botanical and chemical fingerprints.

MATERIALS AND METHODS

Sample preparation

Leaves of *Taxus baccata* was collected from Kew garden, London and *Abies webbiana* was purchased from herbal market, Thanjavur, Tamilnadu, India. Both the plant materials were identified and authenticated by the Botanist (Dr. N. Ravichandran) from Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur. Plant materials were shade dried and milled into fine powder and used for further analysis.

Preparation of extracts

Both chloroform and methanolic extracts were prepared by taking 25 g powdered material of each sample separately with 250 ml of respective solvent in a closed glass container and kept on an orbital shaker at 500 rpm for 3 h at room temperature. The contents were then filtered through Whatman filter paper and the final volume was noted. Both chloroform and methanolic extracts were evaporated to dryness using rotovapor (Make: Buchi, Model R-300) and the dry extract was re-dissolved in respective solvents in the ratio of 10 mg/ml. Chloroform extract was used to carry out proton NMR analysis whereas methanolic extract was used for the phytochemical analysis, UV-Visible scanning, FT-IR, HPTLC and HPLC finger-printing studies.

Quantification of polyphenols

Total phenolic content of methanolic extract of *T. baccata* and *A. webbiana* were analyzed using Folin-Ciocalteu reagent method with some modifications.[29] The extract (100 µl) was added to 250 µl of Folin-Ciocalteau reagent and vortexed for 1 min. Then, 1.0 ml of 5% sodium carbonate solution was added and the mixture is vortexed again for 1 min. A blank was prepared with 100 µl of the solvent (distilled water) instead of the extract. The tubes were incubated at 40°C for 30 min in the dark. The absorbance was read at 720 nm against the blank using Spectrophotometer (Make: Perkin-Elmer). A calibration curve was prepared with standard gallic acid (16 – 100 mg/L, $R^2 = 0.9939$) and used to calculate the total phenolic content of extracts and the results are expressed as gallic acid equivalents (mg GAE / 100 g).

Spectroscopic analysis

UV-Visible scanning of suitably diluted methanolic extract of *T. baccata* and *A. webbiana* was carried out in the wave length range of 200 – 780 nm in a UV-Visible spectroscopy (Make: Thermo Scientific Model: Evolution 201).

For FT-IR spectroscopic analysis, finely powdered raw materials of *T. baccata* and *A. webbiana* were oven dried at 60°C. Two milligrams of the sample was mixed with 100 mg KBr (FT-IR grade) and then compressed to prepare a salt disc (3 mm diameter). The disc was immediately kept in the sample holder and FT-IR spectra were recorded in the absorption range between 400 and 400 cm^{-1} using FT-IR spectrometer (Make: Perkin-Elmer, Model: Spectrum-100).

The 1H NMR spectra of chloroform extract of *T. baccata* and *A. webbiana* (5 mg each) in chloroform-d (Sigma-Aldrich, USA) were acquired using a NMR spectrometer (Make: Bruker Biospin, Switzerland, Model 300 MHz AVANCEII) equipped with a 5mm BBO probe. The experiments were recorded at 298.15 K using the standard pulse sequence library of Top Spin 1.3 followed by processing of the data by using Top Spin 3.2 software.

Chromatographic analysis

HPTLC profile of methanolic extract of *T. baccata* and *A. webbiana* was analyzed on a pre-coated silica gel 60 F-254 (0.2 mm thickness) HPTLC plate (10 x 10 cm, Merck, Germany). Samples (10 µl each) were applied on the plate as 7 mm bands, 15 mm apart from the edges of the plate, with a Camag Linomat V sample applicator. Mobile phase of Toluene: Ethyl acetate: Formic acid (50:40:10, v/v/v) was used for the analysis. The plates were developed to a distance of 80 mm at 25 ± 5°C in a Camag twin trough glass chamber. The saturation time was 30 min and after development, plates were dried in a hot-air oven, viewed in a Camag UV chamber at 254 and 366 nm and the chromatograms were scanned with a Camag TLC Scanner. The Rf values and fingerprint data were recorded using WINCATS software.

For HPLC analysis, methanolic extract of *T. baccata* and *A. webbiana* were purified using membrane filter (Nupore, PVDF syringe filter 0.45 micron) and analyzed using HPLC (Make: Agilent, Model: Infinity 1200). Mobile phase consists of Acetonitril (A) and Water with 2% acetic acid (B) and the gradient conditions were: 70% B during 0 – 10 min, 60% B in 10 – 15 min, 50% B in 15 – 20 min and 70% B in 20 – 25 min with the flow rate of 1 ml / min. Sample volume of 25 ml was injected manually into the C-18 analytical column (Zorbax Eclipse plus, 250 x 4.6 mm and 5 micron) to separate the phyto-constituents and the output was detected at 320 nm during the run time of 25 min.

RESULTS AND DISCUSSION

Botanical characteristics of *T. baccata* and *A. webbiana* can help us to differentiate these herbals. Both the plants are belongs to the same order Pinales, but *T. baccata* has kept under the family Taxaceae while *A. webbiana* comes under Pinaceae. *T. baccata* has shallow root system with extensive horizontal roots and bark is reddish brown in colour, thin and scaly. Branches are long, not whorled and twigs are green and irregularly alternate. Buds are very small and bud scales are dark-brown, rounded, imbricate and closely appressed. Leaves are spirally attached but on lateral shoots, parallel-sided, shortly stalked, mucronate, dark glossy green above, paler and yellowish beneath with two pale stomatiferous stripes. Midrib is prominent on both sides and margins are recurved, tapering to a petiole-

(A) *Taxus baccata* (B) *Abies webbiana*
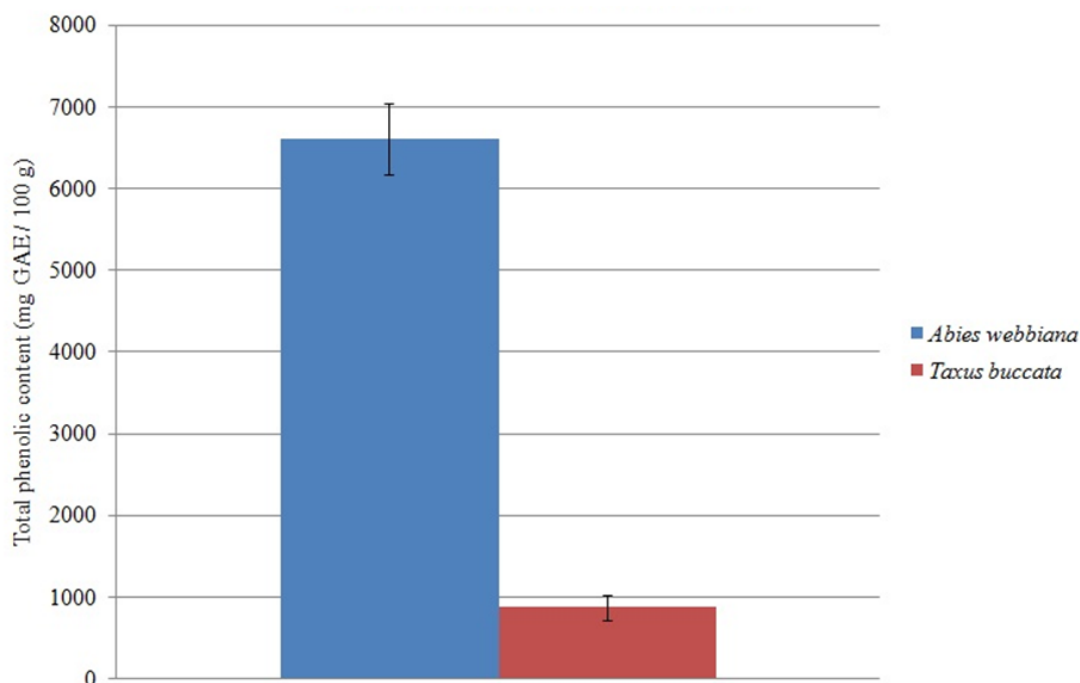Figure 1: Morphology of selected herbal materials.

Figure 2: Total phenolic content of methanolic extract of leaf materials of Taxus baccata and Abies webbiana.

like base. It is normally dioecious and the reproductive structures borne in leaf axils. Seeds are ovoid, smooth and shiny, brown-yellow, with a tough seed coat, partly surrounded by a fleshy red aril[30]. *A. webbiana* is widely distributed on higher ranges of Himalayas region from Kashmir to Assam states in India. It is a tall evergreen coniferous tree grows up to 60 m with strong horizontally spreading branches, young shoots covered with short brown hair. Leaves are simple, densely covering the twigs spreading in all directions, each leaf 1.5-2.3 cm long, aromatic and shiny, midrib in the upper surface is channelled down the middle but raised beneath with two faint white lines on either side of the midrib beneath. Petiole is very short with greyish-brown colour and astringent taste. Cones are blue in colour and the seeds are winged[31].

Between the selected plants, *T. baccata* was reported to be toxic while *A. webbiana* is safe to use in medicine. The poisonous nature of the yew plant except for the fruit has been cited since the second century[4,32]. Taxine, the toxin of *T. baccata*, causes cardiac arrhythmias leading to severe cardiogenic shock[33] and both fatal and non-fatal poisoning with *T. baccata* have been reported[34]. However, taxol from this plant has proven to be useful in cancer treatment and currently it is used in clinical treatment.

Botanically *T. baccata* and *A. webbiana* could be distinguished when they are intact or freshly collected sample because of the obvious differences in their external morphology. But, when they are dried and / or powdered, it is very difficult to differentiate them due to resemblance in appearance. Also, due to similar common name
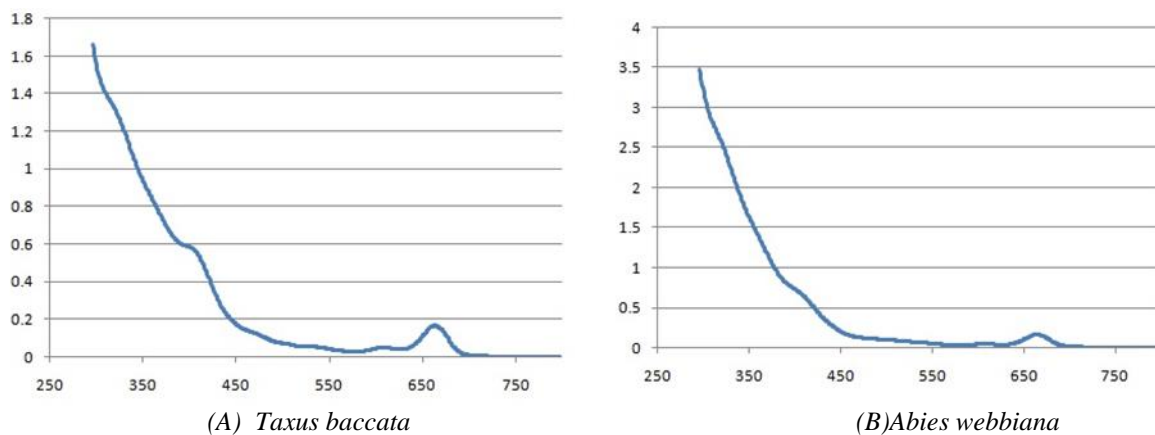
(A) Taxus baccata   *(B)Abies webbiana*

Figure 3: UV-Visible scanning spectrum of methanolic extracts of selected herbals.

(A) *Taxus baccata*   (B) *Abies webbiana*
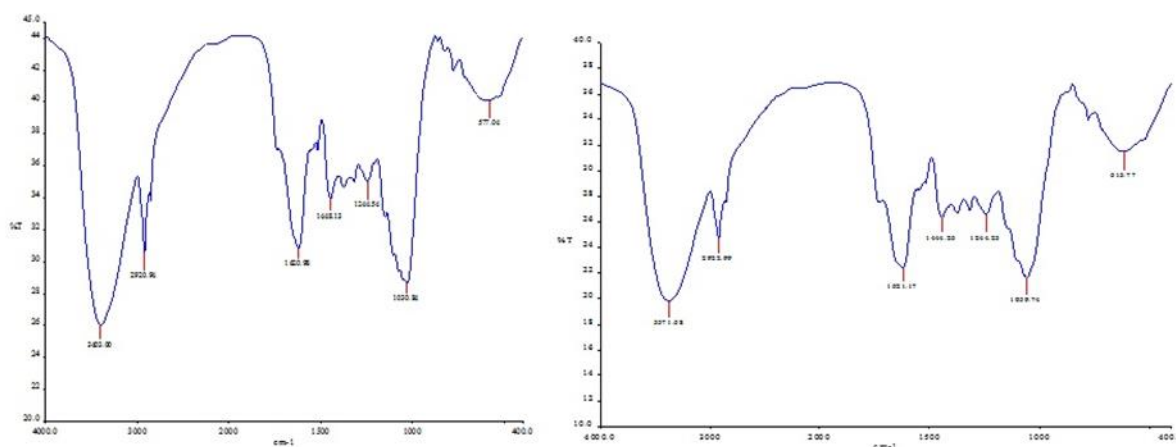
Figure 4: FT-IR spectrum of methanolic extracts of selected herbals.

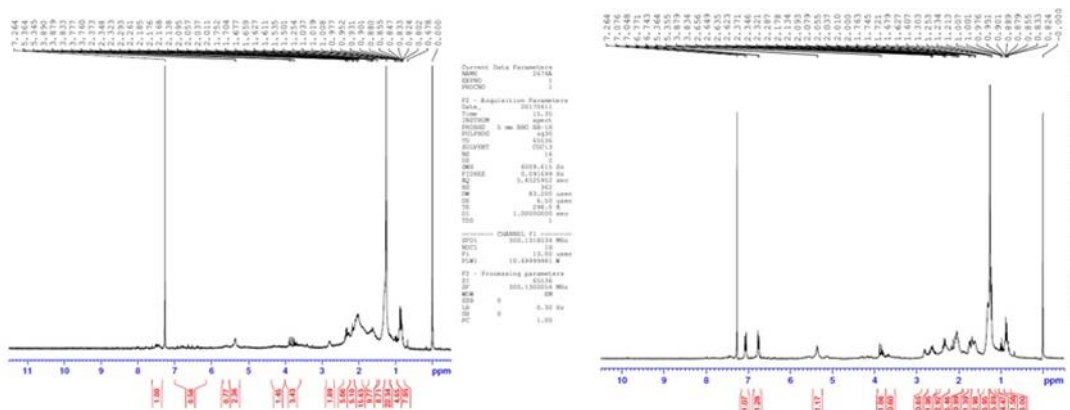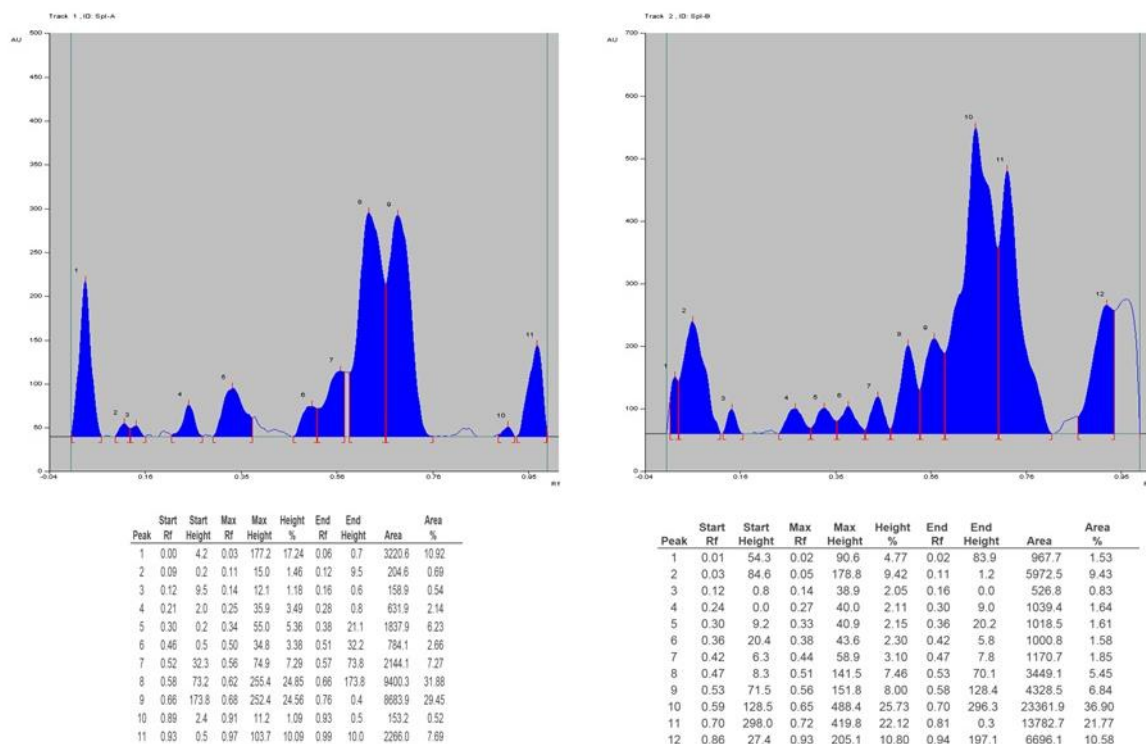(A) *Taxus baccata*   (B) *Abies webbiana*

Figure 5: NMR spectrum of methanolic extracts of selected herbals.

(Talisapatra) in India for both the drugs, there may be a chance for adulteration and misuse of these herbals. Hence, investigation of chemical markers is necessary to differentiate these selected herbals and in this context we have studied the spectroscopic and chromatographic profile of *T. baccata* and *A. webbiana*.

Analysis of total phenolic content of methanolic extract of selected plant materials revealed that *A. webbiana* contained higher level of total phenolic compounds (6301.27 mg GAE / 100 g) when compared to *T. baccata* (977.45 mg GAE / 100 g) (Fig. 2). Higher content of polyphenols observed in methanolic extract of *A. webbiana* of the present analysis provides scientific evidence for its better efficacy noted in Indian traditional medicine. However, it is considered as adulterant or equivalent for the proper drug *T. baccata* in Indian system of medicine. Quantification of total phenolic compounds using Folin's-Ciocaltue reagent (Spectrometric method)

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.00	4.2	0.03	177.2	17.24	0.06	0.7	3220.6	10.92
2	0.09	0.2	0.11	15.0	1.46	0.12	9.5	204.6	0.69
3	0.12	9.5	0.14	12.1	1.18	0.16	0.6	158.9	0.54
4	0.21	2.0	0.25	35.9	3.49	0.28	0.8	631.9	2.14
5	0.30	0.2	0.34	55.0	5.36	0.38	21.1	1837.9	6.23
6	0.46	0.5	0.50	34.8	3.38	0.51	32.2	784.1	2.66
7	0.52	32.3	0.56	74.9	7.29	0.57	73.8	2144.1	7.27
8	0.58	73.2	0.62	255.4	24.85	0.66	173.8	9400.3	31.88
9	0.66	173.8	0.68	252.4	24.56	0.76	0.4	8683.9	29.45
10	0.89	2.4	0.91	11.2	1.09	0.93	0.5	153.2	0.52
11	0.93	0.5	0.97	103.7	10.09	0.99	10.0	2266.0	7.69

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.01	54.3	0.02	90.6	4.77	0.02	83.9	967.7	1.53
2	0.03	84.6	0.05	178.8	9.42	0.11	1.2	5972.5	9.43
3	0.12	0.8	0.14	38.9	2.05	0.16	0.0	526.8	0.83
4	0.24	0.0	0.27	40.0	2.11	0.30	9.0	1039.4	1.64
5	0.30	9.2	0.33	40.9	2.15	0.36	20.2	1018.5	1.61
6	0.36	20.4	0.38	43.6	2.30	0.42	5.8	1000.8	1.58
7	0.42	6.3	0.44	58.9	3.10	0.47	7.8	1170.7	1.85
8	0.47	8.3	0.51	141.5	7.46	0.53	70.1	3449.1	5.45
9	0.53	71.5	0.56	151.8	8.00	0.58	128.4	4328.5	6.84
10	0.59	128.5	0.65	488.4	25.73	0.70	296.3	23361.9	36.90
11	0.70	298.0	0.72	419.8	22.12	0.81	0.3	13782.7	21.77
12	0.86	27.4	0.93	205.1	10.80	0.94	197.1	6696.1	10.58

(A) *Taxus baccata*　　　　　　　　(B) *Abies webbiana*

Figure 6: HPTLC profile of methanolic extracts of selected herbals.
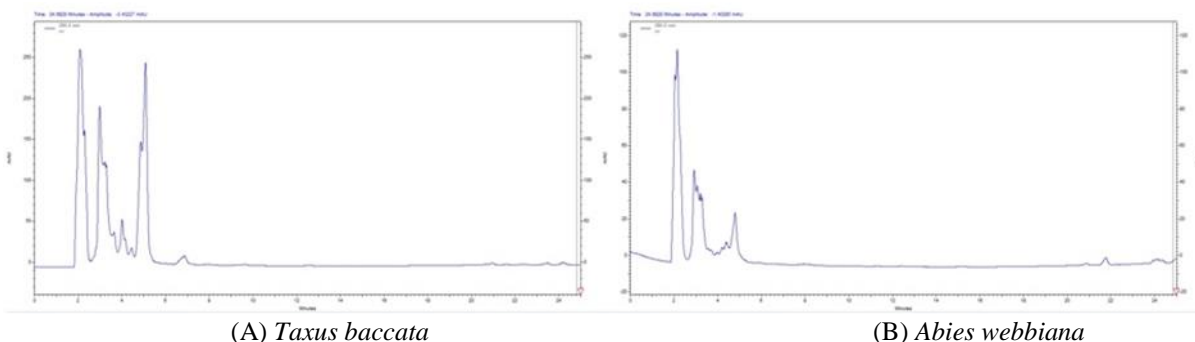
(A) *Taxus baccata*　　　　　　　　(B) *Abies webbiana*

Figure 7: HPLC profile of methanolic extracts of selected herbals.

could help us to differentiate *E. prostrata* from *W. calendulacea*.

Suitably diluted methanolic extracts of *T. baccata* and *A. webbiana* revealed differences at UV-Visible spectrometer scanning (Fig. 3). The absorption of methanolic extract of *T. baccata* was 3.84 at 227 nm, 1.98 at 259 nm, 2.16 at 275 nm, 0.02 at 577 nm and 0.16 at 663 nm. *A. webbiana* extracted showed UV-Visible absorption at 229 nm (3.91), 259 nm (2.13) and 276 nm (2.51). Absorption at 577 and 663 nm are unique only for *T. baccata* and hence UV-Visible absorbance of methanolic extract could be used as one of the useful parameter to differentiate *T. baccata* leaf powder from *A. webbiana*. Application of UV-Visible spectroscopy in determining the herbal fingerprints was explained in detail by Joshi[35]. UV-Vis spectroscopy has been applied to detecting the presence of extraneous food colourants[36].

FT-IR spectroscopic analysis of finely powdered plant materials revealed significant difference at 1000 – 400 cm^{-1} region (Fig. 4). Methanolic extract of *T. baccata* leaf material exhibited transmission of 26.03, 30.51, 30.81, 33.94, 35.04, 28.63 and 40.08% at 3403, 2920, 1620, 1448, 1244, 1030 and 577 cm^{-1} region, respectively. Raw material of *A. webbiana* exhibited percentage of transmission of 19.82% at 3371 cm^{-1}, 24.74% at 2922 cm^{-1}, 22.41% at 1621 cm^{-1}, 26.33% at 1444 cm^{-1}, 26.63% at 1244 cm^{-1}, 21.64% at 1059 cm^{-1} and 31.48% at 613 cm^{-1}. Peaks at 2920, 1620, 1448 and 1244 cm^{-1} of *T. baccata* are found to be similar to that of *A. webbiana*. But, peaks at 3403, 1030 and 577 cm^{-1} were unique for *T. baccata*, which are not available in *A. webbiana*. Similarly, *A. webbiana* exhibited unique peak at 3371, 1059 and 613 cm^{-1}, which are not found in *T. baccata*. Hence, these FT-IR profiles would be helpful to authenticate the selected herbals. Similarly, FT-IR technique was used to identify the adulterants of *Oregano vulgare*[37].

NMR spectroscopy involves the analysis of the energy absorption by atomic nuclei with non-zero spins in the

presence of a magnetic field. The energy absorptions of the atomic nuclei are affected by the nuclei of surrounding molecules, which cause small local modifications to the external magnetic field. NMR spectroscopy can therefore provide detailed information about the molecular structure of a food sample, given that the observed interactions of an individual atomic nucleus are dependent on the atoms surrounding it. NMR spectroscopic results of chloroform extracts of selected plant materials were shown in Fig. 5. Methanolic extract was used for all other analysis like phytochemical analysis, UV-Visible spectroscopy, HPLC and HPTLC, but it can't be analyzed in NMR and hence, chloroform extract was prepared exclusively for NMR analysis. Signals at 7.264, 2.373, 2.348, 2.323, 2.176, 2.095, 2.057, 2.037, 2.011, 1.677, 1.627, 1.254, 1.008, 0.977, 0.952, 0.901, 0.855, 0.833 and 0.824 ppm regions were found to be common for both *T. baccata* and *A. webbiana* extracts. But, *T. baccata* showed characteristic signals at 5.364, 5.345, 3.890, 3.879, 3.833, 3.777, 3.760, 2.293, 2.261, 2.185, 2.168, 2.138, 1.752, 1.704, 1.659, 1.611, 1.535, 1.501, 1.037, 1.019, 0.931, 0.880, 0.847, 0.802 and 0.678 ppm. Likewise, A. *webbiana* exhibited unique NMR signals at 7.076, 7.048, 6.771, 6.743, 5.364, 5.355, 3.879, 3.834, 2.656, 2.649, 2.635, 2.623, 2.287, 2.134, 2.079, 2.000, 1.763, 1.745, 1.721, 1.607, 1.303, 1.234, 1.213, 1.001, 0.889 and 0.879 ppm. Hence, such unique NMR signals could be used to differentiate *T. baccata* from *A. webbiana*. Similarly, Gilard et al.[38] and Vaysse et al.[39] have detected adulterants in herbal dietary supplements using H-NMR technique.

HPTLC is a very simple and rapid analytical method for high potential qualitative characterization and quantitative determination of herbals. Once chemical nature of phytoconstituents were established via HPTLC analysis, it is easy to standardize and validate the herbal products. HPTLC fingerprinting profile of methanolic extract of *T. baccata* showed eleven spots with the Rf value of 0.03, 0.11, 0.14, 0.25, 0.34, 0.50, 0.56, 0.62, 0.68, 0.91 and 0.97 (Fig. 6). On the other hand, twelve spots were observed in the case of *A. webbiana* extract with the Rf value of 0.02, 0.05, 0.14, 0.27, 0.33, 0.38, 0.44, 0.51, 0.56, 0.65, 0.72 and 0.93. Among the detected spots, bands of *T. baccata* with the Rf value of 0.03, 0.14, 0.34. 0.50 and 0.56 were found to be comparable to that of *A. webbiana*, which indicates the presence of similar type of compounds in both the plants. But, bands with Rf value of 0.11, 0.25, 0.62, 0.68, 0.91 and 097 are unique for *T. baccata* while *A. webbiana* exhibited unique spots with Rf value of 0.05, 0.27, 0.38, 0.44, 0.65, 0.72 and 0.93. These unique HPTLC profiles could be useful in distinguishing *T. baccata* from *A. webbiana*. Application of HPTLC fingerprints in determination of quality of botanicals was explained by Nicoletti[40]. Braz et al.[41] have used HPTLC technique to establish quality standards of selected plant species commonly found in the Brazilian market.

HPLC is a popular method for the analysis of herbal medicines because of its easiness and HPLC analysis is not limited by the volatility or stability of the sample compound. In general, it can be used to analyze almost all the compounds in the herbal medicines. Thus, over the past

decades, HPLC has received the most extensive application in the analysis of herbal medicines. HPLC fingerprinting data of methanolic extracts of *T. baccata* and *A. webbiana* was given in Fig. 7. *T. baccata* extract showed a total number of 11 peaks with the retention time of 2.07 (Peak area 24.51%), 2.28 (Peak area 7.17%), 2.98 (Peak area 13.64%), 3.16 (Peak area 2.98%), 3.22 (Peak area 8.94%), 3.63 (Peak area 2.44%), 4.01 (Peak area 5.06%), 4.43 (Peak area 1.61%), 4.86 (Peak area 8.93%), 5.08 (Peak area 18.17%), 5.97 (Peak area 0.46%) and 6.86 min (Peak area 2.05%). In the case of *A. webbiana*, a total number of 9 peaks with retention time of 1.9, 2.0, 2.98, 3.16, 3.20, 3.63, 4.01, 4.43 & 4.5 min were noticed and among which peak at 2.0 min was found to has higher percentage of peak area. Among the HPLC peaks, peak at 2.98, 3.16, 3.22, 3.63, 4.01 & 4.43 min were found to be similar in both *T. baccata* and *A. webbiana* extracts, which indicate the presence of similar type of compounds, but their quantity might varied. Unique HPLC peaks for *T. baccata* are 2.07, 2.28, 4.86, 5.08, 5.97 and 6.86 min while *A. webbiana* revealed unique peaks at 1.90, 2.00 & 4.52 min. So, these unique HPLC peaks could be useful in identification of drug material as either *E. prostrata* or *W. calendulacea*. Deconinck et al.[42] have developed HPLC methods to detect illegal pharmaceutical preparations. HPLC profile data of different basil species (*Ocimum americanum*, *O. basilicum*, *O. citriodorum* and *O. minimum*) were utilized for authentication purpose[43].

CONCLUSIONS

Identification of crude raw drug and authentication of proper herbal is the real challenge in herbal industry, because most of the herbal drugs are sold in powder form and sometimes adulterated with morphologically similar materials / related species plant parts. Hence, development of chemical fingerprints for the identification of proper herbal drug and also to differentiate the adulterants is of paramount importance in herbal industry. In this context, we have developed chemical fingerprints of *T. baccata* and its adulterant *A. webbiana* using phytochemical analysis, spectroscopic methods (UV-Visible, FT-IR and NMR) and chromatographic techniques (HPTLC & HPLC). Results obtained from the present work indicated that the chemical fingerprints might be useful in differentiating *T. baccata* from *A. webbiana*. Experimental results could be useful in quality control process and also to detect the use *T. baccata* or *A. webbiana* in herbal treatment.

ACKNOWLEDGEMENTS

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