

# Mass Spectrophotometric Observation of the Probable Compounds from *Taxus baccata* Linn. (European Yew)

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## ABSTRACT

Highly colored natural products from the powdered leaves and twigs of the plant were successively extracted using hexane and a mixture of chloroform-methanol (1:1). Chromatographic fractions of these extracts were analyzed further. Taxagifine, a 7,9,10-deacetylated taxagifine(1R,2R,3S,4R,5S,6S,8S,10R,11R,12R,15S)-2,3,4,6,11-pentahydroxy-5,15-dimethyl-9-methylidene-14-oxo-16-oxatetracyclo[10.5.0.02,15.05,10]heptadecan-8-yl (2E)-3-phenylprop-2-enoate), a hydroxyl ester (Tricosanyl-16-hydroxy-hexadecanoate), triacontanol-1 and a naphthalene glycoside (1,2,3-tri-O-methyl naphthalene-4-ol- (4→1 $\alpha$ )-glucoside were isolated. Structures of the probable reported compounds were proposed using UV-Vis, FTIR and Mass spectrometric method.

**Keywords:** *Taxus baccata*, Taxagifine, Deacetylated taxagifine, naphthalene glycoside, chromatographic fractions.

## 1. Introduction

*Taxus baccata* Linn. belongs to the family Taxaceae and is an important medicinal plant because of taxoid contents in it. Besides taxoids, several types of other compounds including flavonoid have also been reported to be present in *T. baccata*. Most popular uses of this plant include as traditional medicine such as carminative, cardiogenic, expectorant, antispasmodic, diuretic, antiseptic, emmenagogue, abortifacient, CNS depressant, antipyretic and bronchicum[1]. At present, however it is not used in regular medical practice. This plant is now being extensively used for the isolation of taxol and various taxoids (paclitaxel), as an antitumor drug, antineoplastic agent and is currently approved for the treatment of cancer including ovarian,

breast, lung (non-small and small cell lung cancer), head, neck etc.[2] Also the activity of taxol and taxoids isolated from the species in treating hypersensitivity, leukemia, neurotoxicity, cardiac toxicity and other toxicities appear promising. Literature survey showed (Table-1) that most of the earlier chemical investigations have been concentrated on the isolation of taxol and taxoids (paclitaxel). Besides these, other classes of compounds e.g. glycosides, lignans, carotenoids, mono and polymeric polyphenols and various acids have been reported to be isolated from the plant. Some flavonoid type compounds as phenolic constituents have been isolated and detected. This work reports a systematic study on the non-polar and polar constituents of the leaves and twigs of the plant.

**Table-1:** Chemical Constituents of Leaves and Twigs of *T. baccata* Linn.

Part of the Plant	Chemical constituents	References
Leaves	Taxine (naturally occurring alkaloid mixture)	10
	Taxinin	11
	Taxagifine, Taxaneterpenoid, 19-hydroxy baccatin	4
	Three biflavones, m.p. 310°C, 215°C, 262-2°C	12
	Four biflavones, m.p. 294-6°C, 264-6°C, 258-61°C and 230-5°C	13
	Flavones (m.p. 212°C and 310°C), diflavonoids, totosuflavone.	14
	Betuloside, sequoiaflavone	15
	7,4 <sup>I</sup> , 7 <sup>II</sup> -tri-O-methyl amentoflavone, sucrose, tacosanol.	7
	7,4 <sup>I</sup> , 4 <sup>III</sup> -tri-O-methyl amentoflavone; 7,4 <sup>I</sup> -di-O-methyl amentoflavone; taxicatin, saccharose, raffinose, emulsion, invertin, sucrose	16
	Raffinose	17
Twigs	Taxine (naturally occurring alkaloid mixture)	10
	Triacontyltetracosanoate, $\beta$ -sitosterol	7

## 2. Materials and Methods

The leaves and twigs of Himalayan yew, *Taxus baccata* Linn. were collected from Nepal (high altitude above 2400m.). It was dried and powdered in a grinding machine.

Solvents of origin MERCK, Germany (reagent grade); used for the extractions and further investigations were purified prior to use by distillation at the boiling point of the respective solvent. The dried powder (150gm) was soaked

in distilled n-hexane (ACS, Reag., Merck Germany,  $\geq 99.8\%$ ) at room temperature for 48 hours in an aspirator bottle. The hexane extract was collected and then evaporated to dryness in a rotary evaporator. The hexane extract residue was also collected in a separate container.

A green gummy mass was obtained after evaporation of the hexane extract, designated as H. Fractionation of the extract followed by preparative TLC (glass plates coated with a layer of silica gel, 60 GF<sub>254</sub>, MERCK, activated by drying before use) using chloroform (ACS, Reag., Merck Germany,  $\geq 98.5\%$ ) gave two fractions. One of these fractions (H<sub>1</sub>) on column chromatography eluted by petroleum ether (ACS, Reag., Merck Germany, 40-60°C) followed by mixtures of petroleum ether and dichloromethane (ACS, Reag., Merck Germany,  $\geq 98.6\%$ ) and ethyl acetate (ACS, Reag., Merck Germany,  $\geq 98.5\%$ ) and finally washed with ethyl acetate yielded nine fractions as H<sub>1a</sub>-H<sub>1i</sub>. Yellowish gummy mass H<sub>1b</sub> (18mg) on further column chromatography eluted with petroleum ether, followed by mixtures of petroleum ether and chloroform and finally washed with chloroform afforded compound TB-2 (eluted with petroleum ether).

The residue left after n-hexane extraction was separately extracted with a mixture of methanol (ACS, Reag., Merck Germany,  $\geq 94.6\%$ ) and chloroform at room temperature for 48 hours and then evaporated to dryness in vacuum at a temperature below 45°C that gave deep green viscous mass designated as CM. As it showed unresolved TLC, it was divided into two fractions by triturating with chloroform. The chloroform triturate (blackish) was further divided into eleven sub fractions C<sub>1</sub>-C<sub>11</sub> by column chromatography eluted successively with petroleum ether, mixtures of petroleum ether-chloroform, mixtures of chloroform-methanol and finally washed with methanol. Three of these fractions were further studied.

The yellow gummy mass from chloroform triturate (C<sub>6</sub>) did not show any satisfactory resolution. So, the oily part of the residue was removed by precipitation using methanol. After filtration, the dried residue was chromatographed over a column of silica gel made in cyclohexane (ACS, Reag., Merck Germany,  $\geq 99.8\%$ ) and eluted first with 3% ethyl acetate in cyclohexane and then successively with 5%, 10% and 20% ethyl acetate in cyclohexane and finally washed with ethyl acetate. From that five fractions (C<sub>6a</sub>-C<sub>6e</sub>) were obtained again. One of the five fractions (C<sub>6d</sub>), was further fractionated by column chromatography eluted with 10% ethyl acetate in cyclohexane for purification, which yielded compound TB-5. Decolorization of the dark yellow gummy fraction (C<sub>9</sub>) with charcoal (Sigma Aldrich, 75 microns, 99.97%) from its chloroform solution gave a pale yellow colored material which was fractionated into two parts i.e., ethyl acetate (ACS, Reag., Merck Germany,  $\geq 98.5\%$ ) soluble and insoluble part. Ethyl acetate soluble fraction yielded a needle shaped crystal TB-9 by crystallization using methanol at 50°C which was further purified by washing several times with ice cold methanol.

Fraction C<sub>10</sub> was subjected to column chromatography eluted successively with ethyl acetate, mixtures of ethyl acetate-

methanol and finally washed with methanol (ACS, Reag., Merck Germany,  $\geq 94.6\%$ ). Among the twelve fractions (C<sub>10a</sub>-C<sub>10i</sub>), two fractions were further studied. From fraction C<sub>10f</sub>, a pale yellow amorphous substance was separated out by centrifugation which was purified as fine white needle shaped crystal TB-10 (eluted with 50% methanol in ethyl acetate) by crystallization from ethyl acetate and a few drops of methanol followed by recrystallization from a mixture of petroleum ether and ethyl acetate. Other fraction C<sub>10j</sub>, while concentrated a red crystalline substance was separated out. This substance was purified as fine colorless crystals TB-11 (eluted with 50% methanol in ethyl acetate) by recrystallization from mixture of methanol and ethyl acetate and its purity was checked by TLC in various solvent systems sprayed with aniline-diphenylamine-phosphoric acid reagent.

The further analysis of the compounds were done by Fisher John's electro thermal melting point apparatus, UV-1601(PC)S, Shimadzu, Ultraviolet-Visible Spectrophotometer, Shimadzu FTIR DR-8001 spectrometer and a Hewlett Packard 5890 Mass spectrometer.

### 3. Results & Discussion

#### 3.1. FTIR Analysis

From the IR spectrum of TB-2 (Figure-1), the broad IR absorption band was found at 3425 cm<sup>-1</sup> for  $\nu_{OH}$  and a sharp band at 1736 cm<sup>-1</sup> for  $\nu_{C=O}$ , suggesting the compound to be a hydroxy ester [18]. The presence of an ester group is further supported by the sharp absorption at 1215 cm<sup>-1</sup> and 1176 cm<sup>-1</sup> for -O-C-O stretching. The compound composed of long alkyl chain was evident from the strong bands at 2918 and 2849; 1464 and 1377, and 758 cm<sup>-1</sup> which are characteristic bands for -C-H stretching, bending and rocking vibrations respectively [18].

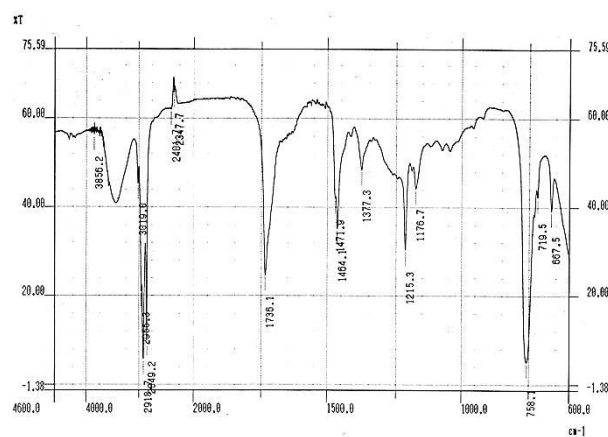
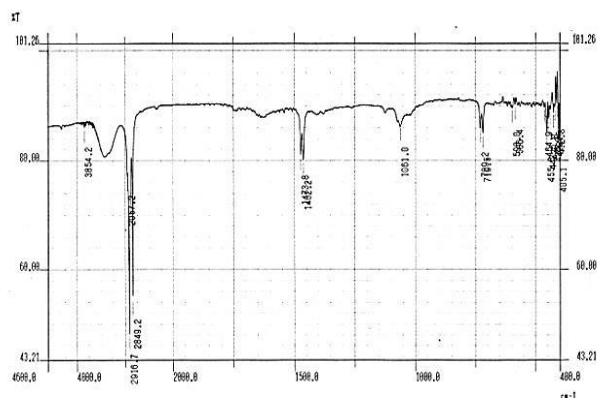


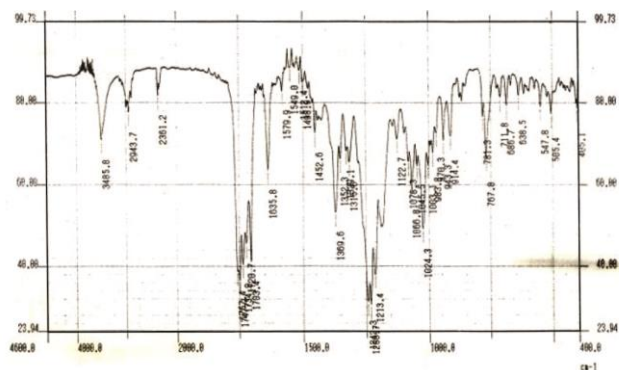
Fig. 1: FTIR spectrum of compound TB-2

IR spectrum of the compound TB-5 (Fig.-2) showed an absorption band at 3425 cm<sup>-1</sup> for hydroxyl group with bands at 2916, 2849 cm<sup>-1</sup>; 1473, 1462 cm<sup>-1</sup> and 729, 710 cm<sup>-1</sup> for the C-H stretching, bending and rocking vibrations respectively. The absorption band at 1061 cm<sup>-1</sup> for C-OH stretching indicated the compound to be a primary alcohol [18].



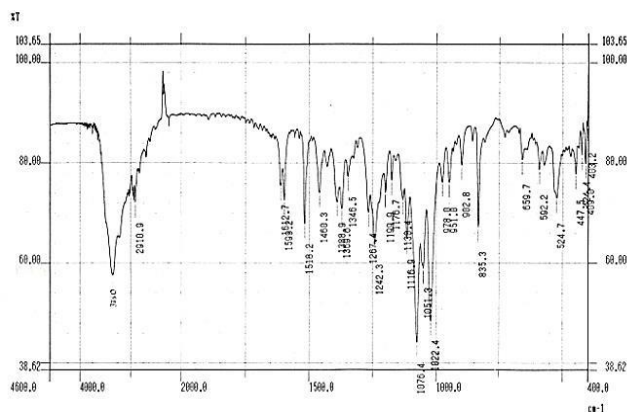
**Fig. 2:** FTIR spectrum of compound TB-5

Again, the IR spectrum of the compound TB-9 (Fig.3) showed absorption at  $\nu_{\max}$   $3485\text{cm}^{-1}$  for O-H stretching exhibited a number of absorptions in the carbonyl region e.g.,  $1757$ ,  $1747$ ,  $1734$ ,  $1720$ ,  $1703$  and  $1635\text{cm}^{-1}$ . The first four C=O absorptions can be easily ascribed to ester carbonyl, the fifth one to a cyclic ketone and the last one to a conjugated carbonyl group [18].



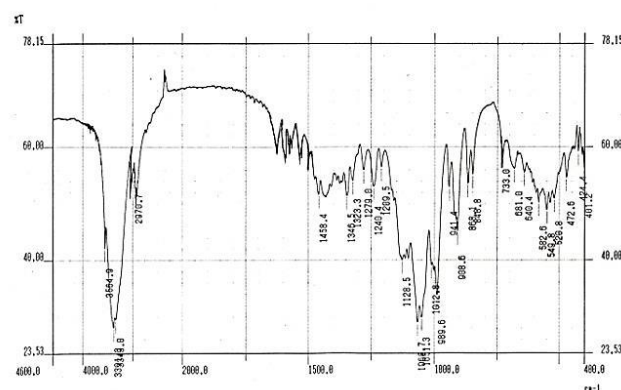
**Fig. 3:** FTIR spectrum of compound TB-9

The IR spectrum of TB-10 (Fig.4) described a sharp -OH absorption at  $3350\text{cm}^{-1}$ . The compound did not show any absorption between  $1612$ - $3000\text{cm}^{-1}$ , only two sharp bands were observed at  $1612$  and  $1599\text{cm}^{-1}$  respectively. Comparison of the IR spectra, solubility behavior and  $R_f$  values of TB-9 and TB-10 clearly indicated that the later was a more polar compound.



**Fig. 4:** FTIR spectrum of compound TB-10

The infrared spectrum of the compound TB-11 (Fig.5) showed a sharp absorption band at  $3564\text{cm}^{-1}$  suggesting the presence of non-hydrogen bonded and broad absorptions at  $3391$  and  $3349\text{cm}^{-1}$  suggesting the presence of hydrogen bonded hydroxyl group [18]. The weak bands at  $2970\text{cm}^{-1}$  and  $1458\text{cm}^{-1}$  indicated the -C-H stretching and bending vibrations respectively, the low intensity suggesting the low proportion of saturated C-H linkage. The other absorption bands at  $3035\text{cm}^{-1}$ (w),  $1625$  and  $1600$ ,  $1590$ ,  $1575$ ,  $1525$  &  $1474\text{cm}^{-1}$  and  $908$ ,  $730\text{cm}^{-1}$  due to =CH-H stretching, -C=C stretching and =C-H out of plane deformation indicating the presence of polynuclear hydrocarbon system like naphthalene in the compound. The bands at  $1069$ ,  $1128$ ,  $1051$ ,  $1012\text{cm}^{-1}$  were indicative of the -CO stretching of hydroxyl compound [18].



**Fig. 5:** FTIR spectrum of compound TB-11

**Table 2:** IR spectral data of the compounds

Compound	IR spectral data $\nu_{\max}$ ( $\text{cm}^{-1}$ )
TB-2	3600(w), 3425(b), 2955, 2918, 2849, 1736, 1471, 1464, 1377, 1215, 1176, 758, 719 and 667.
TB-5	3425, 2957, 2916, 2849, 1473, 1462, 1061, 729 and 710
TB-9	3485, 2943, 1757, 1747, 1734, 1720, 1703, 1635, 1242, 1230, 1213, 1078, 1024 and 767.
TB-10	3350, 1612, 1599, 1518, 1460, 1388, 1369, 1267, 1242, 1116, 1076, 1051, 1022, 835 and 524.
TB-11	3564, 3391, 3349, 3035, 2970, 1625, 1600, 1590, 1575, 1525, 1474, 1458, 1346, 1323, 1279, 1240, 1209, 1128, 1069, 1051, 1012, 989, 941, 908, 868 and 848

### 3.2 UV-Visible Spectrophotometric Analysis

The UV spectrum of the compound TB-9 showed a strong absorption at  $\lambda_{\max}$  283 nm. The UV absorption of TB-9 ( $\lambda_{\max}$  = 283 nm) is identical to that of taxagifine ( $\lambda_{\max}$

=282 nm) [4]. UV spectrum of the compound TB-11 gave absorptions at 232, 276 and 327 nm suggesting the presence of conjugated system similar to naphthalene system [19].

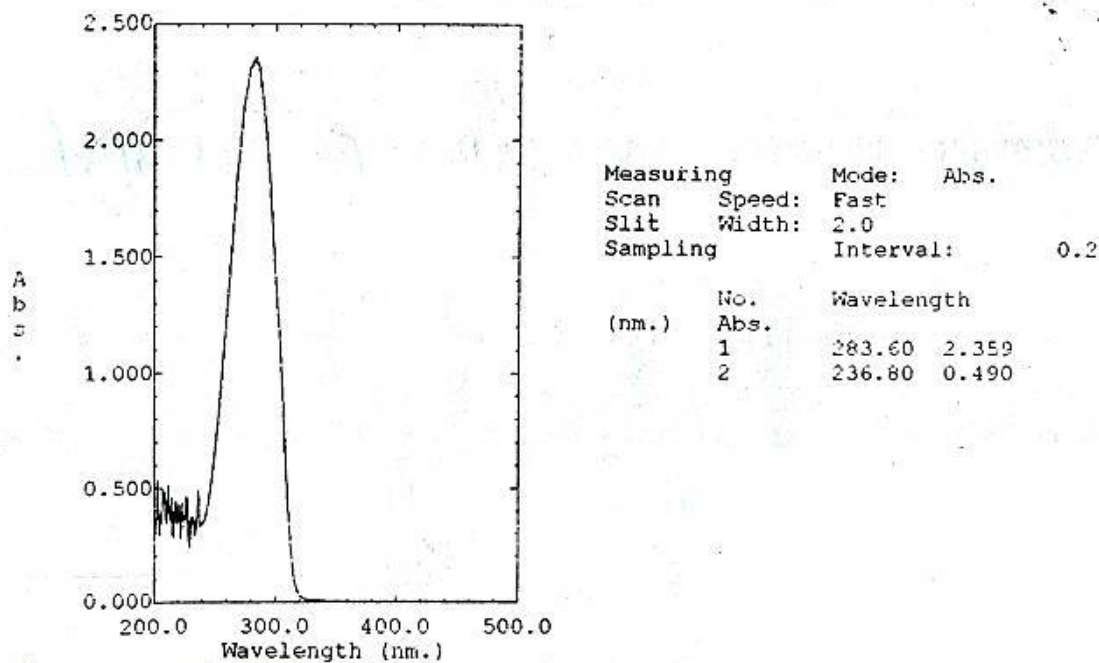
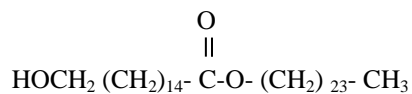


Fig. 6: UV-Visible spectrum of compound TB-9

### 3.3 Mass Spectrometric Analysis

The mass spectrum of the compound TB-2 (Fig.7) showed the molecular ion peak at  $m/z$  608. Considering the compound to be a hydroxy ester with a long alkyl chain the molecular formula can be computed as  $C_{40}H_{80}O_3$ . This is however not very unlikely because the study on earlier works on the acidic constituents of *Taxus baccata* Linn. have revealed the presence of a number of esters of various hydroxy acids [3]. Exercise to explain the mass fragments

based on the presumption of the compound to be a hydroxy ester led to the following structure for the compound.



Scheme 1

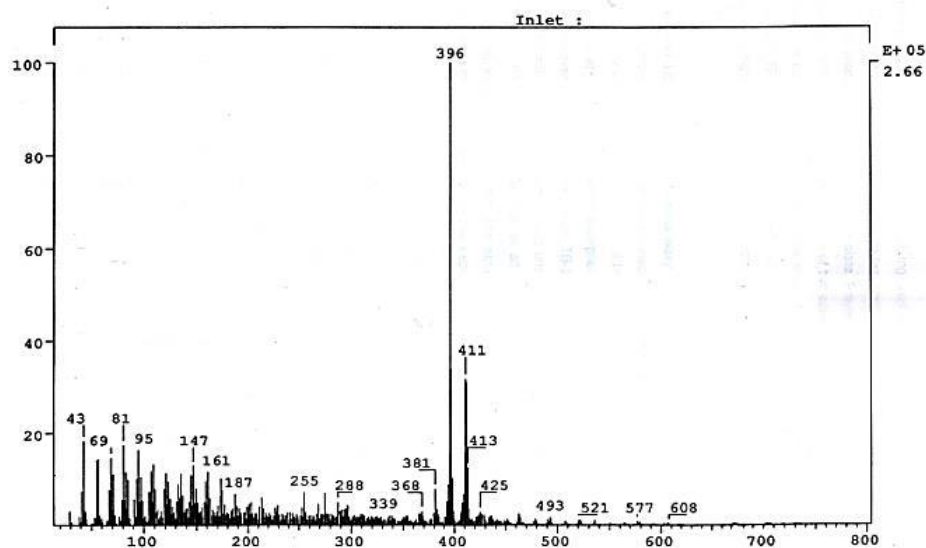
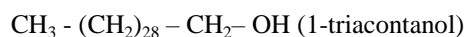


Fig. 7: Mass spectrum of compound TB-2

The mass spectrum of the TB-5 (Fig.8) showed the highest peak at  $m/z$  420. Considering the compound to be primary alcohol [20], it is suspected that the highest mass peak may arise from the molecular ion with loss of a molecule of water ( $M^+ - 18$ ). On this basis the molecular weight of TB-5 could be 438, with the molecular formula as  $C_{30}H_{62}O$  which is the same as that for 1-triacontanol (m.p.  $88^\circ C$ ). Compound TB-5 is thus in all probability 1-triacontanol. However, the base peak at  $m/z$  97 in the mass spectrum can only arise from mass fragments often alkyl group with one double bond ( $C_7H_{13}^+$ ). But the IR spectrum of the compound does not suggest the compound TB-5 to contain any unsaturation. Therefore, the base peak at  $m/z$  97 in the spectrum may arise from an unsaturated compound present in TB-5 as an impurity.



Scheme 2

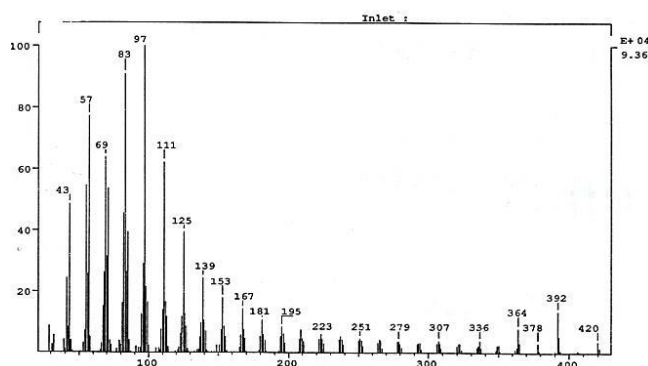
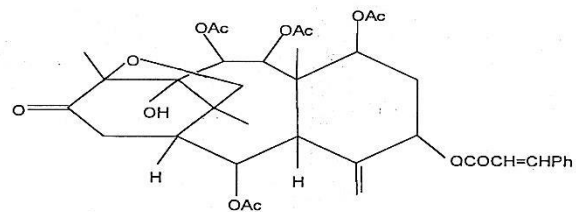


Fig. 8: Mass spectrum of compound TB-5

The mass spectrum of the compound TB-9 showed the molecular ion peak at  $m/z$  696 with base peak at  $m/z$  131 and another peak at  $m/z$  548. An extensive survey of the taxoids isolated from the plants as *Taxus* genus was made. A number of taxoids are known to contain cinnamoyl moiety ( $C_6H_5CH = CHCOO-$ ) which during mass fragmentation is eliminated as cinnamic acid (molecular weight 148). In compound TB-9, similar loss of cinnamic acid from  $M^+$  696 gives the second important peak at  $m/z$  548, confirms the compound to contain a cinnamoyl group. The literature survey also disclosed that the molecular weight of Taxagifine, a taxane derivative isolated from *Taxus baccata*[4] is also 696 and on mass fragmentation loses a molecule of cinnamic acid. Taxagifine is a highly acetylated compound and shows IR absorptions at 1750, 1720, 1640  $cm^{-1}$  with a band at 3450  $cm^{-1}$  for -OH group. The IR characteristics of TB-9 are thus similar to taxagifine[4]. The m.p. of TB-9, 262-264 $^\circ C$  also closed to the melting point of taxagifine, 265-267 $^\circ C$ . The m.p and spectral data e.g. IR and Mass strongly suggest that compound TB-9 to be identical to that of taxagifine[4]. The other important mass fragments at  $m/e$  506, 131, 103 are nicely accommodated by the structure, which unfortunately could not be compared as the total mass spectrum of taxagifine is not available in the literature. Therefore, in all probability TB-9 is taxagifine (III).



Scheme 3

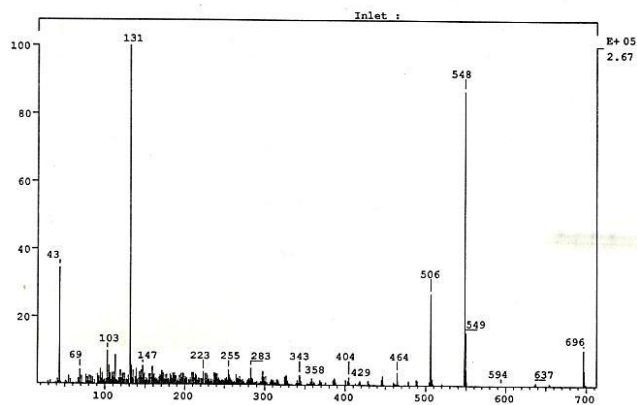
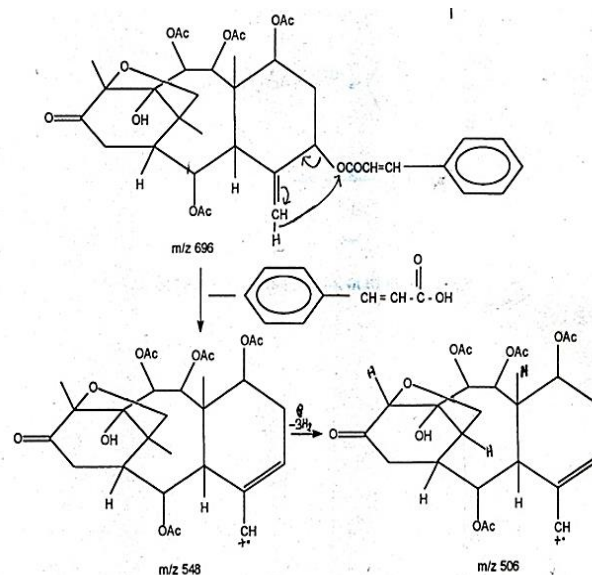
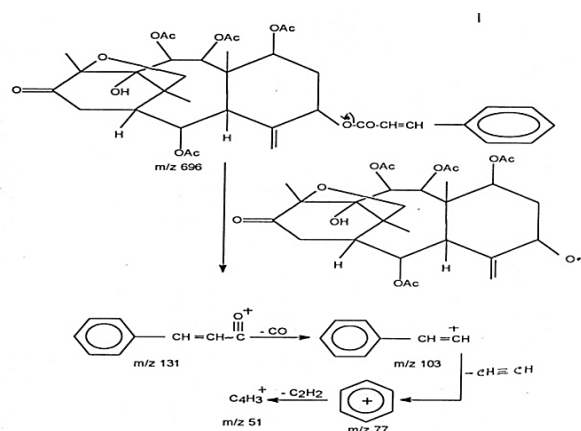


Fig. 9: Mass spectrum of compound TB-9

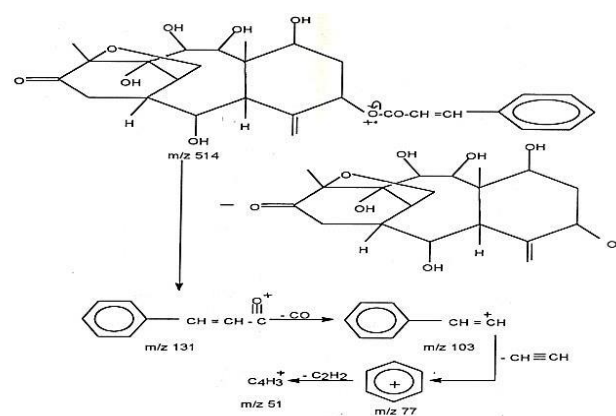


Scheme 6a: Mass fragmentation of compound TB-9 (Mode 1)

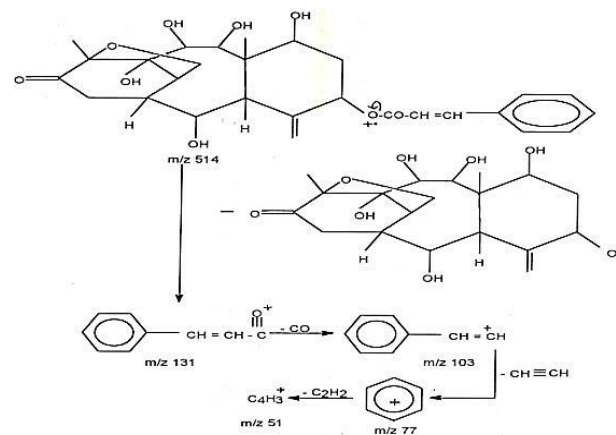


**Scheme 6b:** Mass fragmentation of compound TB-9 (Mode 2)

The mass spectrum of the compound TB-10 (Fig.10) showed the highest mass peak at  $m/z$  514 with the base peak at  $m/z$  131. The base peak at  $m/z$  131 which is also the base peak of TB-9 tends to suggest that TB-10 is also a taxoid like taxagifine, albeit more polar. Various combinations of fragmentations (Scheme 6c-6d) were tried to arrive at a structure for TB-10 based on taxagifine. The difference in the molecular weights of TB-9 and TB-10 (696-514) i.e. 182 can be accounted by deacetylation and removal of a methyl group from taxagifine. This leads to structure TB-10. It may be noted that this type of demethylated C-15 taxoid skeleton has been found to be present in *Taxus Chinensis* [5]. The structure for TB-10 can be used to explain the important mass fragments at  $m/z$  514, 131, 103, 77 and 51 exhibited in the mass spectrum of the compound (Scheme 6c-6d).

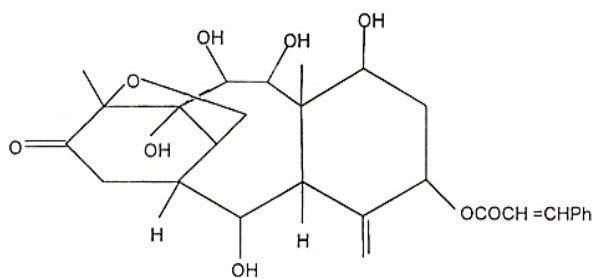


**Scheme 6c:** Mass fragmentation of compound TB-10 (Mode 1)

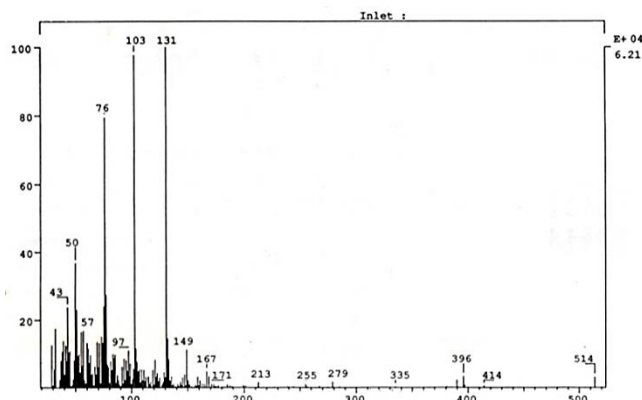


**Scheme 6d:** Mass fragmentation of compound TB-10 (Mode 2)

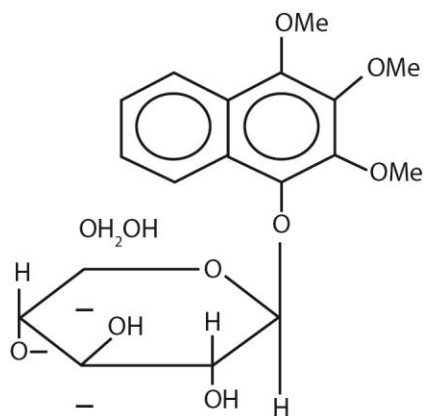
The mass spectrum of the compound TB-11 (Fig.11) showed the highest mass peak at  $m/z$  396 along with another peak at  $m/z$  163 which is the characteristics peak of glucose unit [20]. The mass fragments at  $m/z$  145, 127, 73, 57 and 43 can be explained from fragmentation of glucose unit at  $m/z$  163. Thus the mass spectrum confirmed the presence of sugar moiety albeit in the form of glucose. Considering the highest peak  $m/z$  396 in the spectrum of the compound as molecular ion peak, and that a glucose unit present in it, the molecular formula can be computed as  $C_{19}H_{24}O_9$ . It may be noted that taxicatin (a glucoside of 3, 5 dimethoxy phenol), taxuside (a phenolic glucoside) and a glucoside corresponding to molecular formula  $C_{16}H_{27}O_{11}$  along with other glucosidic compounds have been reported as phenolic constituents of *Taxus baccata* Linn. as well as other *Taxus* species [6, 9]. As the compound gave negative ferric chloride test for phenol, aromatic moiety if present in the compound does not have free phenolic group. Thus several exercises to accommodate the glucose moiety and satisfy the molecular ion peak at  $m/z$  396 results naphthalene system with methoxy group in the compound as the occurrence of this ring system is quite common in natural products. On the basis of the exercises, TB-11 is proposed to have the following structure.



Scheme 4



**Fig. 10:** Mass spectrum of compound TB-10



Scheme 5

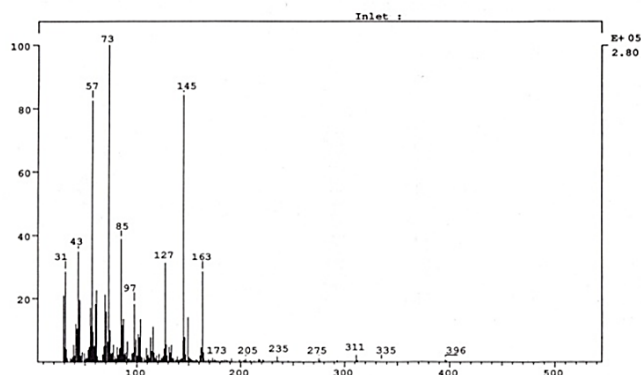


Fig. 11: Mass spectrum of compound TB-11

### 3.4. Melting point and solubility

Compound TB-2 was a colorless waxy substance and melted at 54 °C. It is soluble in petroleum ether, dichloromethane, chloroform and had  $R_f$  0.44 in petroleum ether (40-60 °C) only. Compound TB-5, a white amorphous solid melted at 84-86 °C, was soluble in chloroform, partially soluble in ethyl acetate and methanol. A white needle shaped crystalline compound TB-9 was observed to be melted at 262-264 °C and TLC analysis found  $R_f$  value of 0.26 in a mixture of ethyl acetate and cyclohexane (1:1) and it is soluble in chloroform, ethyl acetate and methanol.

Compound TB-10 was a white needle shaped crystalline compound. TLC examination with methanol: ethyl acetate (1:4) showed the  $R_f$  value 0.39. This compound melted at 134-136 °C. It is insoluble in non-polar solvent but soluble in ethyl acetate when boiled and also soluble in methanol at room temperature.

Compound TB-11 was a colorless fine crystalline substance that melted at 187-190 °C. It is soluble in methanol under hot condition and water at room temperature but insoluble in non-polar solvents.

## 4. Conclusion

This study results in a known compound; taxagifine along with four new compounds 7, 9, 10- deacetylated taxagifine, Triecosanyl-16-hydroxy-hexadecanoate, triacontanol-1 and

a naphthalene glycoside 1, 2, 3-tri-O-methyl naphthalene-4-ol-glucoside from the extract of European Yew. Structure of the isolated compounds have been suggested on the basis of the mass spectrum of the respective compounds which supported by respective IR spectrum. However, suggested compound 7, 9, 10-deacetylated taxagifine is assumed to have medicinal importance which can be further studied with  $^1\text{H}$  and  $^{13}\text{C}$  NMR for its validation.

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