Pharmacognostic studies on *Taxus baccata* L.: A brilliant source of Anti-cancer agents

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Abstract: The present investigation was undertaken to establish standardization profile of *Taxus baccata* L. with the help of pharmacognostic parameters, which is not done before. *T. baccata* (Taxaceae), is native to Europe, is an evergreen needle-leaved tree, growing up to 28 m high. A large number of phytochemicals like taxoids viz. taxusin, baccatin, baccatin, lignans, flavanoids, steroids, paclitaxel and sugar derivatives have been isolated from it. For the treatment of different types of cancer like ovarian and breast cancers, Kaposi's sarcoma and lung cancers Paclitaxel (taxol) has been approved. Paclitaxel is also under clinical trial for remedy of number of other cancers in combination with other chemotherapeutic medications. Pharmacognostical and preliminary phytochemical screening of *T. baccata* will be useful to authenticate and avoid adulteration in the raw material. The diagnostic microscopic characters, physiochemical data and FTIR will be useful in the development of monograph.

Keywords: *Taxus baccata*L, macroscopy, microscopy, FTIR analysis, physio-chemical analysis.

INTRODUCTION

For thousands of year nature has been a source of medicinal agents and a remarkable number of modern drugs have been isolated from this source. A rich heritage of knowledge on protective and therapeutic medicines was presented in prehistoric scholastic work. There was about 13,000 plant species worldwide known to be use as drugs. The trends of using botanicals have increased in the recent year and the active plant extracts are frequently screened for the discovery new medicinal agents (Das *et al*., 1999).

Taxus baccata L. (European or English yew), is an evergreen needle-leaved tree belongs to the family Taxaceae. It is slow growing and long-living tree, reaching maturity only at 70 years (Tutin *et al*., 1964; Thmos and Polwart, 2003; Richard 1985). *Taxus baccata* is native to Europe and also distributed throughout the temperate zones of the northern hemisphere. Traditionally it has been used for making weapon and medicine (Abella, 1996). Classically, there are about eight renowned species of genus Taxus i.e. *T. baccata*, *T. canadensis*, *T. brevifolia*,*T. chinensis*, *T. cuspidata*, *T. globose*, *T. floridana* and *T. wallichiana* (Cope 1998).The genus Taxus has created considerable interest due to the presence of diterpene alkaloids (taxol)(Wani *et al*., 1971).

A large number of phytochemicals like taxoids viz. taxusin, baccatin, baccatin, lignans, flavanoids, sugar derivatives and steroids have been isolated from *T.baccata* (Baloglu and Kingston, 1999; Parmaretal 1999). Two of the isolated lignans from *T. baccata* heart wood lariciresinol and isolariciresinol were revealed to have potent *in vitro* inhibitory effect on tumor necrosis factor (Cho *et al*., 2001a). Since tumor necrosis factor (TNF) is one of the key pro-inflammatory cytokines secreted during the early phase of acute and chronic inflammatory diseases (asthma, rheumatoid arthritis, septic shock). The traditional use of *T. baccata* in inflammatory diseases is due to the presence of lignan derivatives (Cho *et al*., 2001b).

For the treatment of different types of cancer like ovarian and breast cancers, Kaposi's sarcoma and lung cancers Paclitaxel (taxol) has been approved. Paclitaxel is also under clinical trial for remedy of number of other cancers in combination with other chemotherapeutic medications (Rowinsky 1997; Eisenhauer and Vermoken, 1998). Insufficient records have been encountered, for *T. baccata* as traditional medicine in the literature due to the poisonous properties. Its leaves were used as abortifacient, antimalarial, antirheumatic and also used in bronchitis. Dried leaves and barks of *T. baccata* were used against asthma (Bryan 1932; Appendino 1993; Ballero and Fresu 1993; Tekol 1989).

The aim of this study is to establish standardization profile of *T. baccata* with the help of pharmacognostic parameters, which is not done before.

MATERIAL AND METHODS

Plant material

The plant material was collected from Nepal and provided by AK International, Dubai.

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Preparation of extract
Leaves of *T. baccata* were reduced to coarse powder and macerated with methanol for 48 hours. Filtered and filtrate was evaporated under reduced pressure and controlled temperature to obtained dried extract (4.3% w/w). After obtained the extract, preliminary phytochemical screening was done to detect the presence of phytochemicals (Wagner and Bladt, 1996).

Macroscopic evaluation
Macro morphological characters of leaf of *Taxus baccata* sample (color, taste, odor and appearance) were studied and noted.

Microscopic evaluation
Study of transverse section
Cellular arrangement of the leaf of *T. baccata* was examined after making slide of thin transverse section of the leaf. The complete histology was observed under electronic microscope (Shruthi *et al*., 2010).

Study of powder characteristics
Fine powder of leaf of *T. baccata* was used for powder microscopy. The sample was separately treated with 10% chloral hydrate, 50% glycerine and 5% iodine solution (Iyengar 1974; Johansen 1940).

Physio-chemical analysis
For the determination of physico-chemical parameters of powdered leaf sample of *T. baccata* total ash value and moisture content was evaluated (Anonymous 2002).

Moisture content
Moisture content (loss of weight on drying) of leaf powder was determined by weighing 1 gm of powder sample in a silica crucible and then placed the silica crucible in oven at 105°C for a period until constant weight of sample was obtained.

$$\%\ \text{of moisture content} = \frac{\text{Loss in weight of sample}}{\text{Weight of sample}} \times 100$$

Total Ash value
The powdered sample of *T. baccata* about 3 gm was weighed in a silica crucible. For making the sample free from carbon, burn till red hot. Gradually increased the flame and repeat this step until constant value was obtained.

$$\%\ \text{of total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of sample}} \times 100$$

Fluorescence analysis
The fluorescence behavior of the powder drug in the visible light and ultraviolet light were carried out by soaking the powder in different reagent solutions and viewing under the light of required wavelength in a UV chamber (Wallis 1967; Anonymous 1998).

FTIR Analysis
Fine powder of leaves of *T. baccata* was used for FTIR analysis. Infrared spectra were recorded on a Shimadzu FTIR Spectrometer 8000 series, between 4,000-400cm-1.

RESULTS

Phytochemical screening
Preliminary qualitative phytochemical screening of methanolic extract of leaf of *T. baccata* showed the presence of carbohydrates, sterols, amino acids, flavonoids, glycosides, triterponoids and lignains (table 1).

Table 1:Preliminary phytochemical analysis of *T. baccata*

Phyto-constituent	Test	Interference
Alkaloid	Mayer's test	-
	Wagner's test	-
	Hager's test	-
	Dragendroff's test	-
Glycoside	Fehling's test	+
	Keller-killiani test	+
Tannins	Ferric chloride test	_
	Gelatin test	_
	Vanillin-HCl test	_
Resin	Acetone-water Test	_
Flavonoids	Lead acetate test	+
	Sodium hydroxide	+
Lignin	Phloroglucinol test	+++
	Saffranine test	++
Protein	Xanthoproteic Test	++
	Ninhydrin test	++
Carbohydrate	Benedict's test	+++
	Molisch's test	+
	Fehling's test	++
Tri-terpinoid	Salkowski test	+++
Steroid	Vanillin-H_2SO_4test	++
Fats and fixed oil	Copper sulphate test	_

Macroscopic evaluation
Taxus baccata is an evergreen tree attains a height upto 30 m, with a spreading crown. Branches are ascending to drooping with twigs irregularly alternate. The bark is of the tree is reddish-grey or reddish brown. Leaves are needle-like in to two rows, usually curved, acuminate. Margins, slightly in rolled, dark-green, single nerved and narrowing into a short petiole. Flowers are yellowish in color. Seed is hard surrounded by a red fleshy aril, looking like a berry (table 2).

Microscopic evaluation
Study of transverse section
The transverse section of leaf of *T. baccata* showed single layer of upper epidermis covered with thick cuticle.

Beneath the upper epidermis closely packed palisade cells are present. Mesophyll region consist of loosely arranged spongy parenchymatous cell with intercellular spaces between them. Middle portion of the leaf of *T. baccata* contains vascular bundle, which is surrounded by the endodermis cell. The lower epidermis is also single layered (Fig. 1).

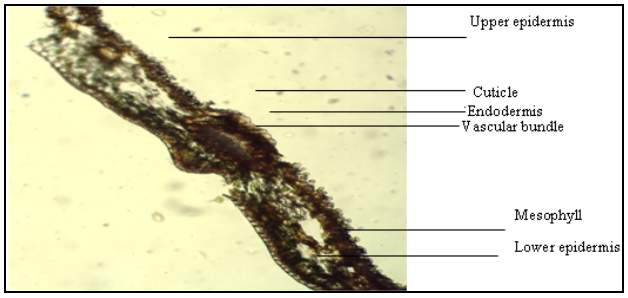

Fig. 1: Tansverse section of the leaf of *Taxus baccata*

Study of powder characteristics

The powder microscopy of leaf of *T. baccata* in different detecting reagents (10% chloral hydrate, 50% glycerine and 5% iodine solution) showed the presence of simple and compound starch granules, simple fiber, fragments of palisade cell, epidermal cell and trichomes (Fig. 2).

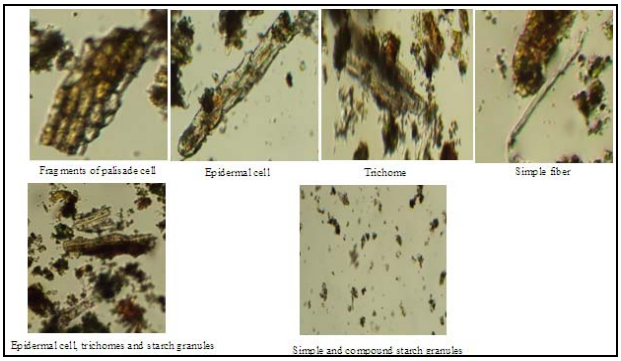

Fig. 2: Powder microscopy of leaf of *T. baccata* in different detecting reagents (10% chloral hydrate, 50% glycerine and 5% iodine solution.

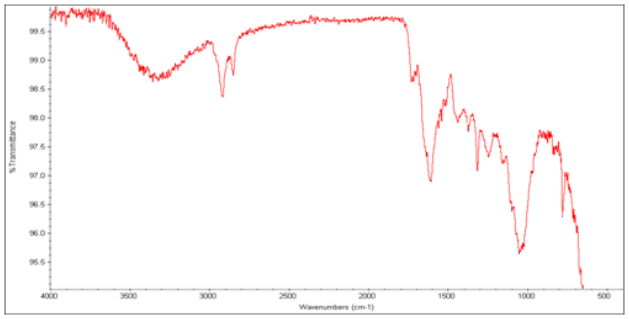

Fig. 3: FTIR Spectra of *Taxus baccata*

Physio chemical analysis

For the determination of adulteration in the sample physiochemical are the important parameter. The total ash value and moisture content of *T. baccata* was tabulated in table 3.

Fluorescence analysis

The characteristic fluorescent colors emitted by the leaf powder of plant samples after treating with various reagents under normal and U.V light were recorded and are presented in table 4.

FTIR analysis

The IR spectrum of *T. baccata* was shown in fig. 3 which manifests prominent transmittance located at 2917, 2847, 1605, 1425, 1300, 1050 and 780cm^{-1}. 2917cm^{-1}peak values indicates the presence of saturated C-H stretching whereas transmittence at 1605 and 1050cm^{-1} attributes C=O and C-O respectively.

DISCUSSION

The process of standardization can be achieved by stepwise pharmacognostic studies as stated above. These studies help in identification and authentication of the plant material. Such information can act as reference information for correct identification of particular plant and also will be useful in making a monograph of the plant. Further, it will act as a tool to detect adulterants and substituent and will help in maintaining the quality, reproducibility and efficacy of natural drugs.

Table 2: Macro morphology of leaf of *T. baccata*

Name of plant	Part Used	Color	Taste	Odor	Appearance
Taxus baccata	Leaf	Green	Slightly Bitter	No Characteristic odor	Leaves are flat, dark green. There is one needle-like leaf per node.

Table 3: Physiochemical analysis of powdered leaf of *T. baccata*

Plant name	Part Used	% w/w	
		Moisture content	Total ash value
Taxus baccata	Leaf	0.012	0.182

n = 3; *dry weight basis

Table 4: Fluorescence analysis of powdered leaf of *T. baccata*

S. No	Powdered crude drug+ reagent	Leaf of *T. baccata*		
		Ordinary light	UV light (254 nm)	UV Light (366 nm)
1	Powder as such	Olive green	Dark green	Black
2	Powder treated with 1N NaOH	Brown	Dark brown	Black
3	Powder treated with Picric acid	Dark green	Dark brown	Blackish brown
4	Powder treated with 50% H_2SO_4	Dark brown	Black	Black
5	Powder treated with 1N HCl	Reddish brown	Dark brown	Black
6	Powder treated with 50% HNO_3	Reddish brown	Orangish brown	Black
7	Powder treated with Acetone	Dark green	Light brown	Black
8	Powder treated with 5% iodine	Orangish brown	Brown	Dark green

The results of the moisture content in *T. baccata* was not high indicates less chances of microbial degradation of the drug during storage, because the excess moisture can results in the breakdown of important constituents by enzymatic activity and which may encourage the growth of yeast and fungi during storage (African Pharmacopoeia 1986). The total ash value in *T. baccata* was 0.182%, since the accepted range was 22%, which implies that the plants have normal complexes of inorganic and organic component (British Pharmacopoeia 1980). The high ash value is the indicator of the mineral contents in the food materials (Anita *et al*., 2006). For the determination of functional groups in the entire drug samples infrared spectroscopic technique was used to judge the reactivity of sample towards therapeutic action and possible efficacy which play an important role in evaluation of drugs. IR spectrometry is more sensitive and selective method than colorimetric method and also a time saving technique to characterized and analyze microorganism (Kogel 2000; Grube *et al*., 2008).

CONCLUSION

Pharmacognostical and preliminary phytochemical screening of *Taxus baccata* leaf will be useful in order to authenticate, standardize and avoid any adulteration in the raw material. The diagnostic microscopic characters, physico-chemical data and FTIR spectra will be helpful in the development of a monograph regarding cancer research.

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