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Research Article

Histochemical Studies of *Boswellia ovalifoliolata* Bal. & Henry – An Endemic, Endangered and Threatened Medicinal Plant of Seshachalam Hill Range of Eastern Ghats of India.

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ABSTRACT

Boswellia ovalifoliolata Bal. and Henry (Burseraceae) is a potential medicinal tree used traditionally in the treatment of ulcers, inflammation, arthritis, obesity and diabetes. The present study aimed at locating and identifying the phytochemical constituents present in medicinally useful parts of *Boswellia ovalifoliolata*. The results indicate that the distribution of secondary metabolites like tannins, polyphenols, crystals and starch grains in various regions of leaf, stem, stembark and root of *Boswellia ovalifoliolata* by using different chemicals or reagents ($FeCl_3$, Iodine solution, toluidine blue reagent and HCl). The results showed that the bluish black, purple or blue, bluish green and dark black colours indicate the presence of tannins, starch grains, polyphenols and crystals respectively in various regions like epidermis, endodermis, midrib, cortex and vascular bundle of leaf, stem, stembark and root of *Boswellia ovalifoliolata*. Histochemical studies are helpful in drug adulteration and systematic Hierarchy of taxon.

Keywords: *Boswellia ovalifoliolata*, histochemical, secondary metabolites.

INTRODUCTION

Boswellia ovalifoliolata Bal. and Henry is an endemic, endangered, globally threatened medicinal taxon belongs to the family Burseraceae[1,2]. This deciduous medium sized tree occurs at an altitudinal range of 250-600 m on Seshachalam hill range of Eastern Ghats of India. Seshachalam hills are habours large number of endemic, endangered, rare, threatened and key stone species due to its vivid geographical conditions and climatic factors which are favourable for the distribution of unique endemic plant wealth[3]. The fresh leaf juice used to prevent throat ulcers[4]. Decoction of the stem bark 10 – 25 ml per day reduces rheumatic pains[5]. The gum obtained from the trunk which is highly medicated, this gum is sold in the local market by the native tribals as Konda sambrani in Telugu language. Small lumps of fresh light yellow coloured liquid oozes out from the stem and hardens on exposure. Amyrins are the chief constituents of the gum together with resin acids and volatile acids. Shade dried gum is powdered dissolved in water and mixed with curd and given orally to cure amoebic dysentery[6]. Gum powder of *Boswellia ovalifoliolata* and *Boswellia serrata* and fruit powder of *Pedalium murex* mixed in equal parts and made into paste and apply externally on the affected part of the testicle to cure hydrocoel. Gum powder mixed with white precipitate of pounded stem of *Tinospora cardifolia* and honey given orally in small quantities (10 ml) two times a day to cure hydrocoel[7]. Equal mixture of gum and stem bark in one tea spoonful given daily with sour milk on empty stomach for a month to cure stomach ulcers[5]. Tribals (Nakkala, Sugali and Chenchu) and local healers of surrounding villages making deep incisions on the main trunk to extract the gum but unknowingly causes damage to immature plants leading to depletion of this species in its natural habitat. Herbal medicines are crude plant drugs used by tribals and rural folk and has also been studied for biological synthesis of silver nanoparticles and antimicrobial activity[8, 9, 10], phytochemical screening[3], quantification of phytochemicals[11], antiulcer activity[12] and antihyperlipidermic activity[13].

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues[14]. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as proteins, lipids, starch, phytin and minerals like calcium, potassium and iron[15]. The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other markers. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice. For example, the presences of calcium oxalate crystals in various plant families have been reported by various scientists[16] reported that the size and shape of calcium oxalate crystals though variable in each species showed enough interspecific differences that may be used for taxonomic references in *Vigna* species. This has been done in other groups of plants such as *Svensonia hyderobadensis*[17], *Cochlospermum religiosum* [18], *Sesbania* species[19], Dioscoreaceae[20], Icacinaceae [21], Nyctaginaceae[22] and Verbenaceae[23]. The

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Table 1: Histochemical analysis of leaf, stem, stembark and root of *Boswellia ovalifoliolata*

S. No.	Reagent	Leaf	Stem	Stembark	Root	Constituents
1.	Toluidine blue	Bluish green	Bluish green	Bluish green	Bluish green	Polyphenols are present
2.	$FeCl_3$	Bluish green	Bluish green	Bluish green	Bluish green	Tannins are present
3.	Iodine	Blue	Blue	Blue	Blue	Starch grains are present.
4.	HCl	Dark black	Dark black	Dark black	Dark black	Crystals are present

Table 2: Fluorescence studies of stembark of *B. ovalifoliolata*

S. No.	Experiment	Visible/ Day Light	UV 254 nm	UV 365 nm
1	Stembark Powder as such	Dark red	Blue	Blue
2	Powder +1N NaOH (Aqueous)	Dark brown	Blue	Blue
3	Powder +1N NaOH (alcoholic)	Thick red	Blue	Blue
4	Powder +1N HCl	Orange	Violet	Violet
5	Powder +50%H_2SO_4	Black	Blue	Blue
6	Powder +Acetic acid	Light yellow	Violet	Violet
7	Powder +Ferric Chloride	Black	Violet	Violet
8	Powder +HNO_3	Yellow	Blue	Blue
9	Powder +NH_3	Dark brown	Violet	Violet
10	Powder +HNO_3+ NH_3	Yellow	Blue	Blue

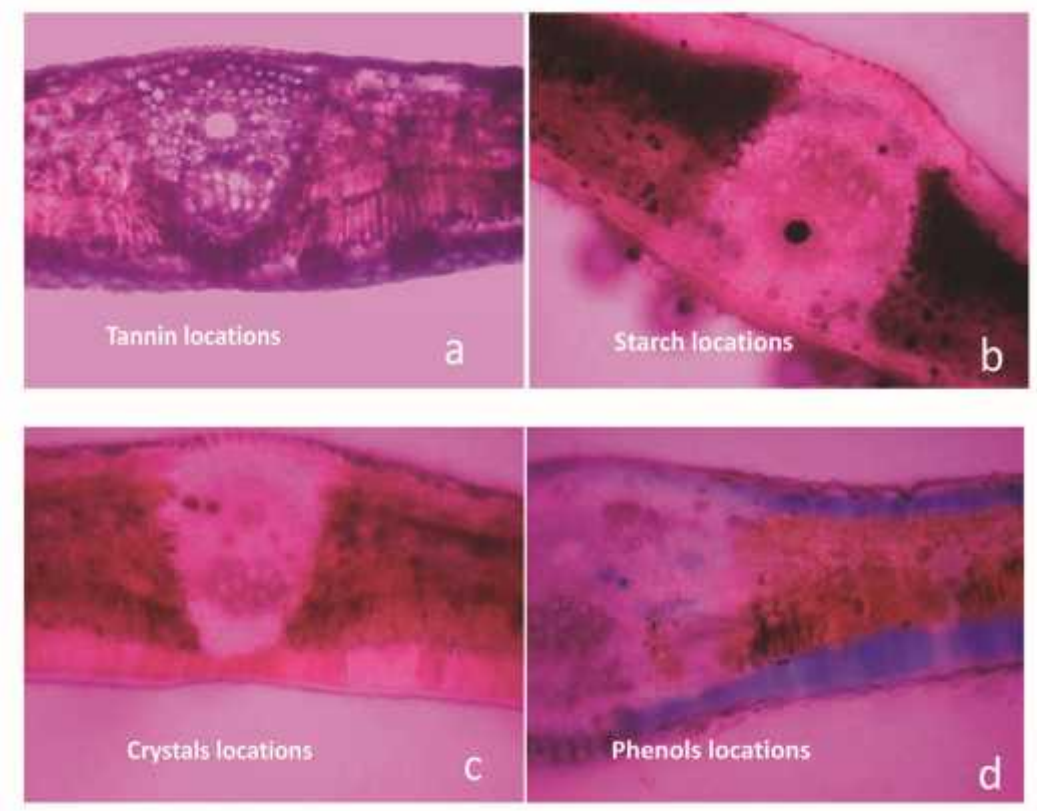

Fig 1: Histolocalization of leaf of B. ovalifoliolata, a) location of tannins, b) location of starch, c) location of crystals and d) location of phenols

biosystematic importance and implications of histochemical features of ergastics, calcium oxalate crystals, nature of tannins and saponins have been investigated in various plants families such as Dioscoreaceae[24], Leguminosae-papilionoideae[25].

Identification of localization of secondary metabolites in plant parts which are using in the preparation of drug is an immense importance to prevent adulteration and also helpful in taxonomic hierarchy. Hence in the present study an attempt has been made to identification and localization of secondary metabolites in various parts of *B. ovalifoliolata*.

MATERIAL AND METHODS

Fresh and healthy leaf, stem, stembark and root of *Boswellia ovalifoliolata* were collected from Tirumala

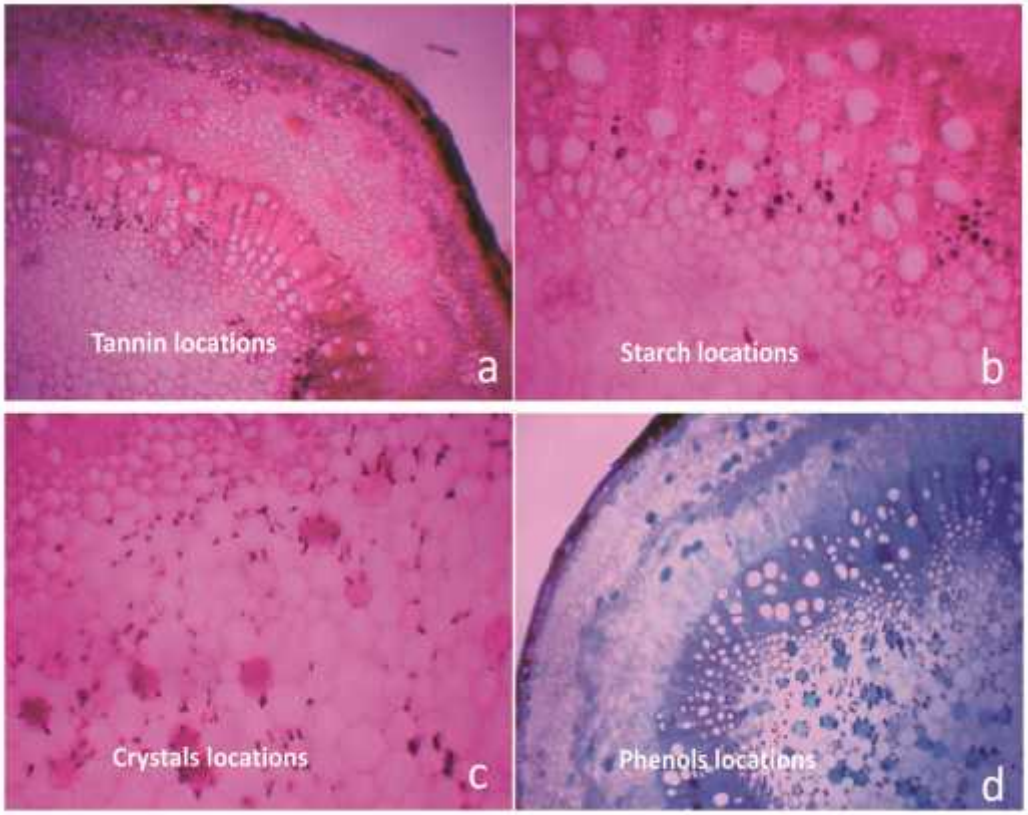

Fig 2: Histolocalization of stem of B. ovalifoliolata, a) location of tannins, b) location of starch, c) location of crystals and d) location of phenols.

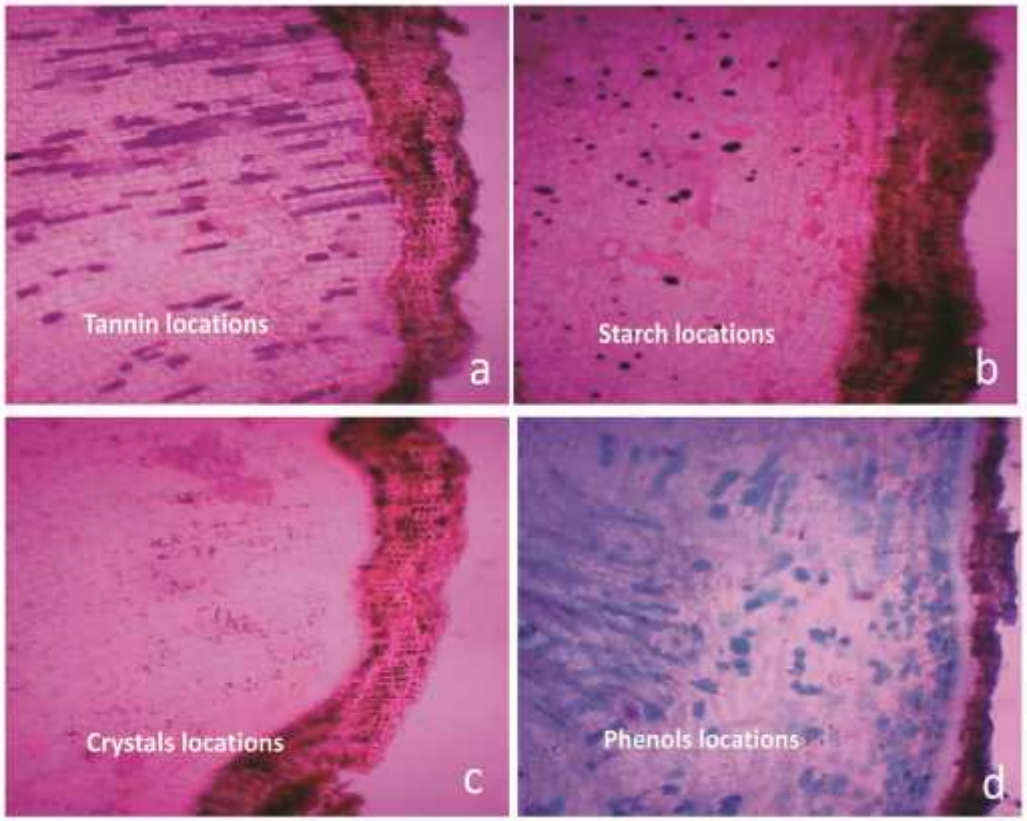

Fig 3: Histolocalization of stembark of B. ovalifoliolata, a) location of tannins, b) location of starch, c) location of crystals and d) location of phenols

hills of Chittoor district, Andhra Pradesh, India during the year December, 2012. These specimens were initially fixed in FAA (1:1:18) glacial acetic: 40% formaldehyde: 70% ethanol (v/v) for 48-72 h after 72 h transverse (T.S) sections were taken using a rotary microtome (RMT-30). Anatomical staining was done by initially staining with few drops of alcian blue for 5 min and counter stained with safranin solution for 2 min. The slides were treated with $FeCl_3$, Iodine solution, tolidune blue reagent and HCl for identification of polyphenols, tannins, crystals and starch grains. Photomicrographs of the anatomical features were then taken from the slides using Nikhon Labhot 2 microscopic unit[26].

Fluorescence studies: The fluorescence studies were carried out as per the method of Bhattacharya and Zaman[27]. A small quantity of the stembark powder was placed on a grease free clean microscopic slide and added 1-2 drops of the freshly prepared reagent solution, mixed by gentle tilting the slide and waited for 1-2 min. Then the slides were placed inside the UV-viewer chamber and viewed in day light short (254 nm) and long (365 nm) ultraviolet radiations. The colours observed by application of different reagents in different radiations were recorded.

RESULTS AND DISCUSSION

Histochemical color reactions were carried out through transverse sections of leaf, stem, stembark and root of *Boswellia ovalifoliolata* (Table-1). Various secondary metabolites are identified in leaf with treatment of different reagents. The presence of tannins was indicated by the development of bluish black colour, when treated with ferric chloride ($FeCl_3$). The tannins were found mainly in the parenchyma tissue of the midrib region, whereas the starch grains were indicated by the development of blue color or purple, when treated with iodine solution. The starch grains were located in the upper and lower epidermis and paranchymatous region of midrib. The presence of polyphenols were indicated by the development of bluish green color, when treated with toluidine blue reagent. The polyphenols were found surrounding the vascular bundle sheath. The presences of crystals were indicated by the development of dark black color, when treated with HCl, the crystals were present in the midrib region and vascular bundles (Fig-1).

Fig-2 showed that the histochemical studies of stem, the presence of tannins was indicated by the development of bluish black color, when treated with ferric chloride ($FeCl_3$). The tannins were presented in endodermis, cortex and secondary phloem regions. The starch grains were indicated by the development of blue color or purple, when treated with iodine solution found in epidermis, cortex and vascular bundles. The polyphenols were found mainly in the endodermis and cortex region, which were indicated by the development of bluish green colour, when treated with toluidine blue reagent. The presences of crystals were indicated by the development of dark black color, when treated with HCl the crystals were present mainly in the cortex.

The histochemical studies of stembark deals with the presence of tannins were indicated by the development of bluish black color, when treated with ferric chloride ($FeCl_3$). The tannins were presented in endodermis and cortex regions. The starch grains were indicated by the development of blue color or purple, when treated with iodine solution which are found in epidermis and cortex. The polyphenols were found mainly in the endodermis and cortex region indicated by the development of bluish green colour, when treated with toluidine blue reagent. The presences of crystals were indicated by the development of dark black color, when treated with HCl the crystals were present mainly in the cortex (Fig-3).

The histochemical analysis of roots showed the presence of tannins in cortex and vascular bundle region which were indicated by the development of bluish black color, when treated with ferric chloride ($FeCl_3$) (Fig-4). The polyphenols were identified in the cortex region by the development of bluish green color, with treatment of toluidine blue reagent. The presence of crystal indicated by the development of dark black color, when treated with HCl, which are present mainly in the cortex. The starch grains were indicated by the development of blue or purple colour, when treated with iodine solution and found mainly in the cortex and vascular bundle regions.

Tannins contribute property of astringency i.e. fasten the healing of wounds and inflamed mucous membrane. Primarily phenolic compounds are of great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics, bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress which may cause many diseases, including cancer, cardiovascular problems and ageing.

Starch is mainly stored within xylem parenchyma ray tissue of underground organs. This type of storage tissue can be considered to be expensive in terms of resource allocation as ray parenchyma cells of wood are living and non-photosynthetic and require a high metabolic demand to be both created and maintained [28, 29]. Toluidine blue is a cationic dye that binds to negatively charged groups. An aqueous solution of this dye is blue, but different colors are generated when the dye binds with different anionic groups in the cell for example, a pinkish purple colour will appear when the dye reacts with carboxylated polysaccharides such as pectic acid; green, greenish blue or bright blue with polyphenolic substances such as ligning and tannins; and purplish or greenish blue with nucleic acids[30]. Plants store glucose as the polysaccharide starch, it can be separated into two fractions-amylose and amylopectin. Amylose forms a colloidal dispersion in hot water, whereas amylopectin is completely insoluble. The structure of amylose consists of long polymer chains of glucose units connected by an alpha acetal linkage. Amylose in starch is responsible for the formation of a deep blue colour in the presence of iodine. The iodine molecule slips inside of the amylose coil. When added the Iodine-KI reagent to a solution or other materials blue colour is present. If starch amylose is not present, then the colour will stay orange or yellow.

Fluorescence Analysis: The fluorescence characteristics of stembark powder with different chemical reagents were

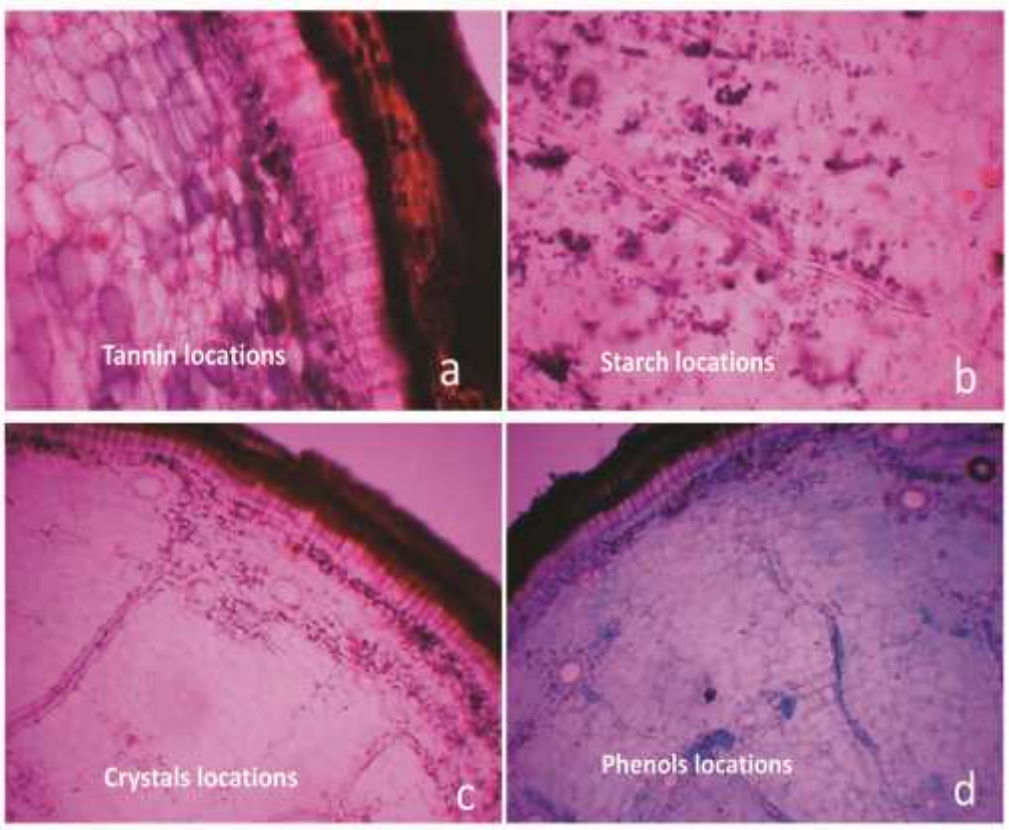

Fig 4: Histolocalization of root of B. ovalifoliolata, a) location of tannins, b) location of starch, c) location of crystals and d) location of phenols

summarized in Table-2. Although a change in color was observed by the addition of various reagents under day light, none of the reagents induced any fluorescence to the stembark powder under both short and long UV radiations. Under UV light dark brown and black colors were prominent. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents showed fluorescence in the visible range in the day light. The natural products (Alkaloids) produce fluorescence in UV light but do not produce fluorescence in visible day light. If the substances themselves are not fluorescent, they often be converted fluorescent derivatives or decomposition products by applying different reagents[31,32].

The histochemical studies and fluorescence studies of *Boswellia ovalifoliolata* are useful to supplement the information with regard to its botanical identification and drug standardization. Moreover, it also helps in distinction from other allied species and adulteration.

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