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Research Article

HISTOCHEMICAL LOCALIZATION OF SECONDARY METABOLITES IN DIFFERENT TISSUES OF *AVICENNIA MARINA* COLLECTED FROM DIU COAST, GUJARAT

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ABSTRACT
It is essential that any crude plant part of mangrove plants used for medicinal purpose needs to be subjected to botanical inspection to avoid side effects in the body. However, there are no detailed botanical studies on mangrove plant parts to help in the proper identification. The morphological, anatomical and phytochemical analysis is the best solution for identification of such crude plant parts. Therefore, histochemical localization of secondary metabolites such as alkaloids, phenols, tannins, lignins, anthocyanins and anthoquinones were done in the leaf, stem and root tissue of *Avicennia marina*. This was done with an aim to know which types of secondary metabolites are present in the plant tissues. It was observed that the phenols and tanins get deposited in the parenchyma and collenchyma cells. Alkaloids get deposited only in the phloem and medullary cells. Lignins get deposited in the xylem, phloem and medullary cells. Anthocyanins get deposited in the sclerenchyma cells and anthoquinones get deposited in parenchyma, collenchyma and cambium cells of plant tissue. From the above analysis it was concluded that by localizing secondary metabolites in plant tissue, one can easily know which type of secondary metabolites are present in the plant tissue. These can help in distinguishing plant material from each other. The repeated use of plant material for treatment of specific human ailment can be done.

Keywords: *Avicennia Marina, Secondary Metabolites, Histochemistry, Diu, Natural Habitat*

INTRODUCTION
Pharmacological uses and medicinal importance of mangroves is known to local people since ancient time. The medicinal uses of mangroves have a wide spectrum. Different species of mangroves are used for different medicinal purposes traditionally. In 19th century Nadkarni (1908) and Kirtikar and Basu (1918) have given a detailed account of mangrove species for their medicinal importance and mode of action of few species. Naskar and Guha Bakshi (1987) stated that mangroves are widely used in Sundarbans for curing diseases on traditional basis. Most of the work on mangrove medicine is on the species such as A. officinalis, A. marinavar. acutissima, Acanthus ilicifolius, R. mucronata, Excoecaria agallocha, Salvadora persica, Ipomoea pes-caprae and others. Naskar and Chakraborty (1984) have identified many species of mangroves of Sundarbans delta from village folk and folk literatures for medicinal uses. Nadkarni (1908) reported that the root, bark, tender shoot and leaves contains active metabolites which has got medicinal property. The members of Rhizophoraceae, Avicenniaceae and Acanthaceae family show presence of astringent property (Sathe *et al.,* 2016). According to Das *et al.,* (2018) fruits of Avicennia officinalis are used against boils. Rhizophora mucrcnata is used in diaebetes, haemorrage, dysentry, leprosy (Sur *et al.,* 2015). Ceriops tagal is used in haemorrhage and malignant ulcers (Rastogi and Mehrotra 1991). Kandelia candel is used to treat diaebetes as it has antidiabetic potential (Shettar, and Vedamurthy 2017). Bruguiera gymnorrhiza, is used in eye medicine as astringent (Rahman *et al.,* 2013). Sonneratia alba is used in haemorrhage and swellings (Ghani 2003). Avicennia officinalis, is used in small pox, Boils, abscesses skin parasites and wounds. Acanthus ilicifolius is used in snake bite, asthma, cough, Nerve tonic, dressing boils (Sathe *et al.,* 2016).. Excoecaria agallocha is used in epilepsy, ulcers, leprosy, and poisoning (Mondal *et al.,* 2016).

However, Need of today's scenario is identification of plant metabolites from mangrove species, which are useful for human benefits. Natural plant metabolites are the excellent bioresources to cure infectious diseases, preparation of dyes, cosmetics and other pharmaceutical products. Therefore, identification of metabolites synthesized in various mangrove plant parts should be screened and used for treatment of various ailments. It is essential that any crude plant part used for medicinal purpose needs to be subjected to botanical inspection to avoid side effects. However, there are no such data available for mangrove plants for the proper identification. The morphological, anatomical and biochemical level analysis is the best solution for identification of such mangrove plant parts. Hence, in present studies an attempt is done to identify secondary metabolites present in plant parts of Avicennia marina through histochemical localization process and confirmation through biochemical screening.

MATERIALS AND METHODS

Sample collection

Present research work was carried out at the natural habitats of Avicennia marina at the coastal region of Diu. Plant samples (leaves, stem and roots) were collected from the natural habitat following random sampling method.

Histochemical localization of secondary metabolites

Histochemical analysis was performed on transverse sections of fresh stem obtained from the different plant species. Transverse sections of stem were treated with various different solutions to precipitate out the secondary metabolites present within the plant tissue. T. S. of stem was treated with mayer's reagent to precipitate out alkaloids in stem tissue. T. S. of stem was treated with phloroglucinol prepared in 20 % HCl to stain lignin in stem tissue. T. S. of Stem was treated with 10 % Potassium dichromate to precipitate out phenolic compounds present in stem tissue. T. S. of Stem was treated with 5 % FeCl3 to precipitate out tanins present in the stem tissue. T.S. of stem was treated with ammonium hydroxide solution to precipitate out anthocyanins present in stem tissue. T.S. of stem was treated with benzene solution to precipitate out anthoquinones present in stem tissue. Image documentation was performed using a digital camera (MICAPS) coupled on an LABOMED microscope. Images were taken in Light field microscopy using MICAPS image-capturing software.

Preliminary Qualitative Analysis

Ethanol extract of leaves **(**1 ml) was mixed with 2 ml of 5% ferric chloride and incubated for 5 min. The Formation of dark blue or greenish black color indicated the presence of tannins in the extract. Ethanol extract of leaves **(**2 ml) was mixed with 2 ml of distilled water and shaked in graduated cylinder for 15 min. lengthwise. The formation of 1cm layer of foam indicated the presence of saponins. Concentrated HCL (2 ml) was added to the ethanol extract of leaves (2 ml) with few drops of mayer's reagent. The formation of green or white colored precipitates indicated the presence of alkaloids. Ethanol extract of leaves (2 ml) was mixed with 2N sodium hydroxide (1 ml) solution to observe formation of yellow color which indicated presence of flavanoids. Ethanol extract of leaves (2 ml) was mixed with 3 ml of chloroform and 10% ammonium hydroxide solution to observe formation of pink color which indicates presence of glycosides. Ethanol extract of leaves (1 ml) is mixed with concentrated sulphuric acid (1 ml) to observe formation of red color which indicates presence of quinines. The ethanol extract of leaves (1 ml) was mixed with 10 % potassium dichromate to develop reddish brown color which indicates presence of phenol. Ethanol extract of leaves (0.5 ml) was mixed with 2 ml of chloroform and concentrated sulfuric acid. The formation of brown color in solution indicate presence of terpenoids. The ethanol extract of leaves (2 ml) was mixed with few drops of 0.2% ninhydrine reagent and heated for 5 min. the formation of blue color indicates the presence of aminoacids. The ethanol extract of leaves (1 ml) was mixed with 10 % Sodium hydroxide (1 ml) to form yellow color which indicates presence of coumarins.

RESULTS AND DISCUSSION

It is a common belief that drugs of natural origin are safe than chemically synthesized drugs. Therefore, demand of crude drugs is increasing at present days (Hoareau and DaSilva 1999). Crude drugs made from mangrove plant parts are used traditionally to cure various diseases. However, due to lack of ability in identification of raw plant materials of mangrove plants deleterious effect of herbal medicines on human health has been observed. Hence, current scenario demands for correct botanical identification of plant parts used for medicinal purpose. Hence, In the present studies, *Avicennia marina* plant parts (Stem, root and leaf) were selected as a model system to determine the types of secondary metabolites present in plant tissues. Transverse section of each plant parts were stained with different secondary metabolites precipitating solutions. This was done to localize secondary metabolites *in vivo* in plant cells. Even, anatomical attributes of the plant parts are useful in identifying plant species (Sen et al 2010, Pace et *al.,* 2011). It is believed that the production and concentration of bioactive compounds is higher in mature tissue than that of juvenile tissue. Hence in present studies mature leaves, stem and roots were selected.

Further, Histochemical localization of secondary metabolites such as alkaloids, phenols, anthocyanins, tannins, anthroquinones and lignins were localized in transverse section of each plant part. These compounds were selected because each of these secondary metabolites have medicinal value. For example, Alkaloids are found to possess important biological activities such as antioxidation, antimalarial, anti-bacterial, antifungal, anthelmintic, cardiotonic, anticonvulsant, anti inflammatory, analgesic activity, anti-cancer activity, anti-diabetic, antihyperglycemic and antitumor activity (Ziegler and Facchini (2008); Kainsa *et al.,* 2012; Marella *et al.,* 2013; Frick *et al.,* 2005; Nassiri, 2013; Herman and Herman, 2013). Phenolic compounds have wide spectra of medicinal uses (Santos *et al.,* 2003). Polyphenols found in plants are involved in defense against ultraviolet radiation and defense against pathogens. Plant polyphenols act as antioxidants. Epidemiological studies and associated meta-analyses have strongly suggested that long term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Pandey and Rizvi, 2009). Tannins are polyphenols found in plants that play crucial role in anticarcinogenic, antimutagenic, antioxidant and antimicrobial activities (Chung *et al.,* 1998). Coumarins are found to have multi-biological activities such as anti-HIV, anti-tumor, anti-hypertension, anti-arrhythmia, anti-osteoporosis, pain relief, and preventing asthma and antisepsis (Hao *et al.,* 2008). Lignins are polymer of phenylpropanoid subunits, they are found to have antioxidant activity in humans (Domenek *et al.,* 1999). The use of anthocyanins in human ailments is supported by anecdotal and epidemiological evidences. It is being used in remedies for liver disfunction, hypertension, vision disorders, microbial infections, diarrhea and diverse other health disorders (Lila, 2004). Anthraquinones has a broad range of bioactivities such as anti-inflammatory, cathartic, anticancer, diuretic, antimicrobial, phytoestrogen, and vasorelaxing activities, which are useful in clinical application of many diseases (Chien *et al.,* 2015).

Histochemical localization of secondary metabolites suggested that secondary metabolites get accumulated in specific layer of stem tissue. Its concentration changes in each and every plant species. Alkaloids present in plant tissues were precipitated using saturated solution of picric acid. The picric acid is acidic in nature when it binds with basic natured alkaloids it forms yellow coloured salts. Therefore, when transverse section of each plant sample was treated with saturated solution of picric acid, alkaloids present in plant cells was precipitated. This resulted in the formation of yellow colour staining in plant cells containing alkaloids. It was observed that yellow coloured salts were precipitated in xylem, phloem and medullary cells of plant tissue. Phenols present in plant tissues were precipitated using 10% Potassium dichromate solution. Phenols when bind with Potassium dichromate it gives reddish brown precipitates. Therefore, when transverse section of each plant sample was treated with 10% Potassium dichromate solution, phenols present in plant cells was precipitated (Gabe, 1968; Andreia *et al.,* 2013). This resulted in the formation of reddish brown color staining in plant cells containing phenols. It was observed that reddish brown coloured precipitates were found in Parenchyma cells of cortex region and

Pith region of plant tissue. Tannins present in plant tissues were precipitated using $Fecl_3$ solution (Yadav *et al.*, 2014). Tannins form dark green precipitates with iron (Fe) compounds. Hydrolysable tannins are derived from gallic acid are known as gallitannins. These tannins when treated with ferric chloride produces brown color. Condensed tannins are derived from the flavonols, catechins and flavan-3, 4-diols. These tannins when treated with ferric chloride produces dark green color. Therefore, when transverse section of each plant sample was treated with $FeCl_3$ solution, tannins present in plant cells were precipitated. This resulted in the formation of dark green to brown colour staining in plant cell layers. It was observed that dark brown coloured salts were found in the epidermis, collenchymas, parenchyma and endodermis cells of plant tissue.

Lignins present in plant tissues were determined using phloroglucinol-HCL solution. The cinnamaldehyde end groups of lignins when bind with phloroglucinol it gives reddish violet color in acidic condition (Jensen, 1962; Gahan, 1984). Therefore, when transverse section of each plant sample was treated with phloroglucinol-HCL solution, Lignins present in plant cells appeared reddish violet in color. It was observed that Lignins were present in the vascular tissues of plants. It was present in the walls of xylem elements and phloem tissue. Anthocyanins present in plant tissues were detected using ammonium hydroxide solution. Anthocyanins when interacts with ammonium hydroxide it gives green precipitates (Johansen 1940). Therefore, when transverse section of each plant sample was treated with ammonium hydroxide solution, Anthocyanins present in plant cells was precipitated. This resulted in the formation of green color staining in plant cells containing anthocyanins. It was observed that green coloured precipitates were found in parenchyma, Medullary cells of plant tissue. Anthraquinone when interacts with mixture of benzene and ammonium hydroxide solution to gives yellowish orange precipitates (Chen *et al.*, 2015). Therefore, when transverse section of each plant sample was treated with mixture of benzene and ammonium hydroxide solution, Anthraquinones present in plant cells were precipitated. This resulted in the formation of yellow color staining in plant cells containing anthraquinones. It was observed that yellow coloured precipitates were found in parenchyma, collenchyma and cambium cells of plant tissue.

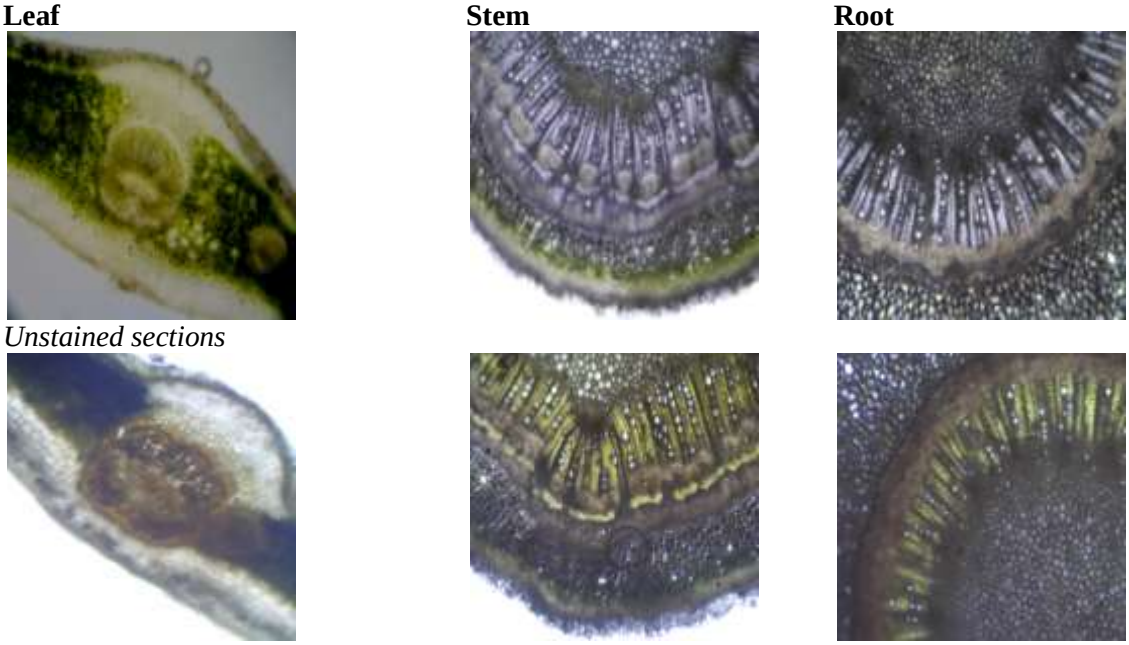
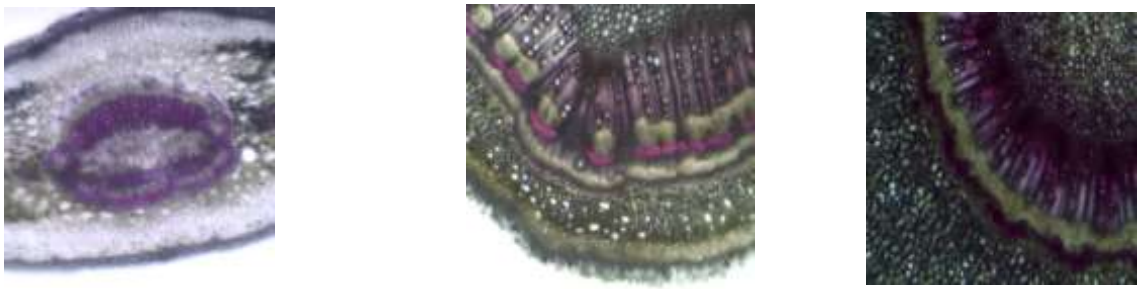


Figure 1: represents the histochemical localization of secondary metabolites in leaf, stem and aerial root cells of *Avicennia marina*

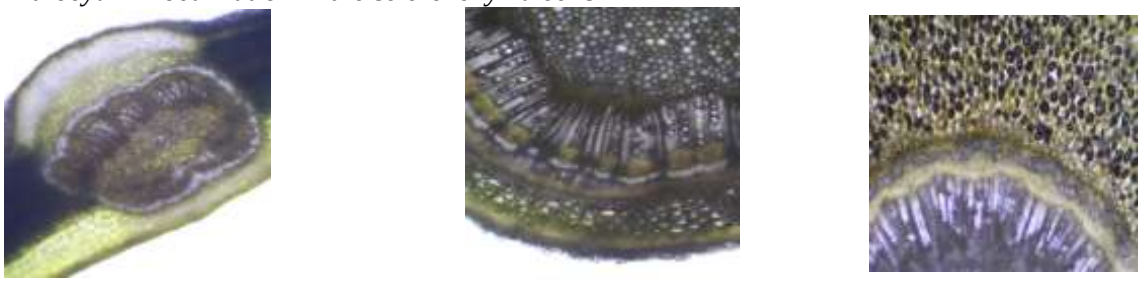


Lignin localization in the xylem, phloem and medullary cells

Tannins localization in the parenchyma and collenchyma cells

Phenol localization in the parenchyma and collenchyma cells

Anthocyanin localization in the sclerenchyma cells



Anthraquinone localization in the in parenchyma, collenchyma and cambium cells

Figure 1: represents the histochemical localization of secondary metabolites in leaf, stem and aerial root cells of *Avicennia marina*

These studies were also supported by preliminary phytochemical screening tests. There was remarkable presence of alkaloids, phenols, tannins, and quinones in the leaves extract it also showed the presence other primary and secondary metabolites such as diterpenes, terpenoids, coumarin, and glycosides in the ethanol extract. The presence of alkaloids, flavonoids, aminoacids, carbohydrates, alcohol, sugar, lipid contents are also reported in previous studies done on *Avicennia marina (*Khafagi *et al.,* 2003). Bark, leaves and fruit of *Avicennia marina* have been used in traditional medicine for the treatment of skin diseases, rheumatism, small pox, ulcers and fodder for livestock. (Fauvel *et al.*, 1993; Bandaranayake 2002). It also possesses antimalarial and cytotoxic activity (Miles *et al.,* 1998). It is a good source of alcohol, amino acid, carbohydrate, fatty acid, hydrocarbons, inorganic salts, minerals, phytoalexins, carboxylic acid, steroids, tannins, triterpenes, vitamins, iridoid glucosides and fatty acids (Bandaranayake 2002; Hogg & Gillan 1984; Kanig & Rimper 1985).

Table:1 represents preliminary phytochemical screening of secondary metabolites from leaf extract of *Avicennia marina collected from natural habitat of Diu coast*

No	**Phytochemical test**	Results
1	Alkaloids	++
2	Glycosides (Carbohydrates)	++
3	Saponins	++
4	Proteins	++
5	Phenols	++
6	Tannins	++
7	Coumarins	++
8	Quinones	-
9	Flavanoids	+
10	Terpenoids	-

CONCLUSION

From the above analysis it has been concluded that the histochemical localization of secondary metabolites can be utilized to ensures quality, safety, efficacy and repeated use of mangrove plant part for preparation of crude drug. The correct identification of a medicinal raw plant material of mangrove in terms of botanical species can be done by image documentation of morphological and anatomical (histochemically localized secondary metabolites in T.S. of plant tissue) characters.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest regarding this publication in the journal.

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