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Histochemical Investigation of *Syzygium cumini* L (Skeels)

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Abstract: The present work is under taken with a view to analyze, similarities and dissimilarities, anatomical, microscopically, physicochemical. These plants are commonly available and medicinally useful in this geographical area and this study would form a foundation for understanding the pharmacological and therapeutically effectiveness of these varieties. One of such resources is traditional medicines. Systematic screening of them may result in the discovery of Secondary metabolic compounds location and quantity in plant organs. This Research Article Histochemical investigation of *Syzygium cumini* these plants have many traditional medicinal used.

KEY WORDS: Histochemistry, traditional Medicine, Secondary metabolites, *Syzygium cumini* L (Skeels)

Introduction

Several published reports demonstrate the use of histochemistry to locate essential oil, such as the localization of citral accumulation in Cymbopogon citratus (E. Lewinsohn etal 1982) ,where the aldehyde- specific Schiff's reagent was used to detect the monoterpene aldehydes neral and geranial (citral), and the lipid stains Sudan red and Nile blue were used to locate essential oil in leaves of Salvia aurea (G. Serrato-Valenti etal 1997) Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues. Starch deposition occurs widely in the plant body, but the particularly common places of its accumulation are seeds, the parenchyma of the secondary vascular tissues in the stem and root, tubers, rhizomes and corn (V. B. Kadam, 1999) Starch and proteins are the principal ergastic substances of the protoplast (E. Kuster etal 1996). Tannin is the heterogeneous group of phenol derivatives, usually related to glucosides. Tannins are particularly abundant in the leaves (xylem) of many plants (V. B. Kadam, 1996).Saponins are the rare occurrence. Fats are widely distributed in the plant body and they probably occurs in small amount in every plant cell(W. Seifriz.,1936). Fats are common reserve material in seeds, spores and embryos in meristematic cells. Glucosides are the degradation product of the carbohydrates. Alkaloids are the

degradation product of protein. Many plants contain medicinally important secondary product.(M. L. Dhar,1938). In the particular, many plants never previously examined are now being screened for possible pharmacological activity and for active constituents such as alkaloids, saponins, flavanones, tannins etc.

Medicinal Uses

Syzygium Cumini plant has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties. Most of the plant parts are used in traditional system of medicine in India. According to Ayurveda, its bark is acrid, sweet, digestive and astringent to the bowels, anthelmintic and in good for sore throat, ronchitis, asthma, thirst, biliousness, dysentery, blood impurities and to cure ulcers (Kirtikar and Basu, 1975). In Unani medicine system the ash of leaves is used for strengthen the teeth and the gums, the seeds are astringent, diuretic, stops urinary discharge and remedy for diabetes and the barks showed good wound healing properties (Nadkarni, 1954).

Myrtaceae is a plant family widely used in folk medicine in different countries and *Eugenia* and *Syzygium* are among its most important genera. Species of this family are often used for several medicinal purposes, including the treatment of diarrhea and pain (Caceres, *et.al.,* 1993). Experimental data also suggest the action of these species on inflammatory processes, respiratory diseases (Muruganandan, *et.al. 2001*), and allergic disorders (Kim, *et.al.,* 1998). The presence of polyphenols, gallic acid, ellagic acid derivatives (Timbola and Szpoganicz, 2002), tannins (Son K, *et.al.,* 1998), and glycosylated flavonoids (Timbola, *et.al.* 2001) has been reported in *Syzygium* species. We extended the previous observation that *Syzygium Cumini* leaf extracts contain flavonoids (Timbola, *et.al.* 2002 and Ramirez RO, Roa CC Jr, 2003). Showed a correlation between the anti-inflammatory activity and the content of the total phenolic compounds in the extracts of *Cumini*. Our results on the antiedematogenic effect of the *Syzygium Cumini* extract also support the earlier observation of (Slowing, *et.al1996).*and (Middleton, 1998) that the presence of flavonoid glycosides may be associated with the anti-inflammatory activity of a methanol.

Syzygium cumini is a medicinal plant, whose parts were pharmacologically proved to possess hypoglycemic, antibacterial, anti-HIV activity and anti-diarrhea effects. (Bhuiyan, *et. al.,* 1996; Kusumoto, *et al.,* 1995; Indira and Mohan, 1993; Ravi, *e*). Slowing *et.al.,* (1994) and Muruganandan *et. al.,* (2001) reported the anti-inflammatory activity of leaf and barks.

Histochemistry and Material Methods:-

Histochemistry combines the techniques of biochemistry and histology in the study of the chemical constitution of cells and tissues. The importance of histochemistry has decreased as IHC methods have developed. However, the breadth of knowledge for many histochemical methods is such that they are still used in decision-making. Temporary and permanent mounts of sections were employed for the test of histochemical studies. For study of isolated different tissues, small pieces of material were macerated in

Jeffery's fluid (Johansen, 1940). Micro chemical tests were performed following methods described by Johansen (1940) and Gurr (1965)

Table –1. Histochemical tests performed are listed below

Sr. No	Erastic Content	Chemical Test	Reference:
1	Starch	Iodine, Potassium iodide soln. (IKI Solution.)	Johansen (1940) and Gurr (1965)
2	Protein	Potassium Ferro cyanide, Glacial acetic acid	Johansen (1940)
3	Tannins	10% ferric chloride solution (aq)	Johansen (1940) and Gurr (1965)
4	Saponin	Conc. Sulphuric Acid	Johansen (1940)
5	Fats	Sudan III Sudan IV	Johansen (1940)
6	Glucosides	Phloroglucinol in 90% alcohol+20% hydrochloric acid	Johansen (1940) and Gurr (1965)
7	Alkaloids	a) Idonine solution	Johansen (1940)
		b) dil. Nitric a cid	Johansen (1940)

Result and Discussion

Histochemistry:

Histochemical localization in different organs of the taxa under study was made, using methods described elsewhere. The initial presentation gives details about the occurrence of erastic content or secondary metabolites, Viz starch, protein, fat, tannin, saponin, glucoside and alkaloids in leaves and stem.

1) Starch:

Starch is the principal ergastic substance of the protoplast. Starch is composed of long chain molecules, whose basic units are anhydrous glucose residues of the formula $C_6H_{12}O_5$. Starch has an ordinary arrangement of molecule and, therefore, shows optical anitsotropy and double refraction. In starch granules the molecule is radially arranged, therefore, in polarized light a cross pattern is seen. The morph metric Variation of starch grain is so extensive that they may be used taxonomically and pharmaconostically up to a limited extent (Kuster, 1956).

Starch deposition occurs widely in the plant body, but the particularly common places of its accumulation are seeds, the parenchyma of the secondary vascular tissue in stem and roots, tuber, rhizome and corms.

In the present work, for the taxa under study, starch was present in leaves and stem of all the taxa, *Syzygium cumini* Linn (Table 2 and Plate 1)

2) Protein:

Protein are the major constituents of the living protoplast, but they also occur as temporarily inactive erastic substance, Erastic protein is knows as a storage material and is found deposited in amorphous and / or crystalline forms. Like starch and cellulose, crystalline protein combine crystalline and colloidal properties, therefore, the individual units of this material are spoken of as crystalloids (meaning crystal like) rather than as crystals. This is also present in all the taxa under investigation. Protein were observed in the leaves and in the stem of Syzygium *cumini* Linn (Table 2 and Plate 2)

3) Tannin:

Tannin is a heterogeneous group of phenol derivatives, usually related to glucosides. Tannins are particularly abundant in the leaves of much plant; in the xylem, in the testa of seeds and in pathological growth like galls (Kuster, 1956; Spelich, 1939). No tissue, however, appears to lack tannins entirely. They may be found in meristematic cells too. Sometimes tannins containing cells are conspicuously associated with a vascular tissue terminates beneath storage tissue or secretary cells of nectarines. The monocotyledons are notably poor in tannins (Sperlich, 1939). Tannins also show distributions, occurring mostly in epidermis, mesophyll cortical as well as parenchymatous tissue, associated with conductive tissue. Tannins were observed in the leaves of and in stem also *Syzygium cumini* Linn (Table2 and Plate 3)

4) Saponin:

The saponin are of rare occurrence and wherever present, they apparently remain to one or two organs.Saponin were observed in the mid-rib parenchyma of leaves and cortex and pith parenchyma of stem *Syzygium cumini* Linn. Saponin were observed in the cells of leaves and of Stem of *Syzygium cumini* Linn (Table 2, Plate 4)

5) Fat:

Fat are widely distributed in the plant body, and they probably occur in small amounts in every plant cell. The term fat may be used to described not only the fats proper (that is, ester of fatty acids with glycerol), but also related substances grouped under the name of lipids (Seifriz, 1936)

As protoplast inclusion, fats are common reserve material in seeds, spores and embryos in meristematic cells and occasionally in differentiated tissue of the vegetable body (Sharp, 1934) .They occur as solid bodies or, more frequently, as fluid droplets of various size either dispersed in the cytoplasm or aggregated in large masses fatty substance are thought to be elaborated directly by the cytoplasm and also by leucoplast. In taxa under study, fat was found in leaves and stem of the, *Syzygium cumini* Linn (Table 2 and Plate 5)

6) Glucoside:

Glucosides are the degradation production of carbohydrates glucosides were observed in the epidermis, pith parenchyma of leaves vascular bundles and scattered cells of medullary ray of stem *Syzygium cumini* Linn (Table 2 and Plate 6)

7) Alkaloids:

Alkaloids are degradation of protein they were investigated by using two methods, namely; Mayer's reagent and Wagner's reagent. In Mayer's reagent alkaloids were observed in the scattered cells of mesophyll of leaves and pith parenchyma of stem .In wanger's reagent, alkaloids were found in the cells, of leaf and Stem of *Syzygium cumini* Linn (Table 2 and Plate7 & 8)

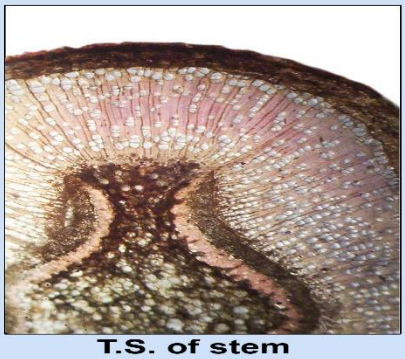
Table 2 Histochemical test for fresh section of leaves and stem of *Syzygium cumini* Linn

Sr. No.	Ergastic Content	Reaction		Localization	
		Leaves	Stem	Leaves	Stem
1	Starch	$^{+}$ve	$^{+}$ve	Upper and lower epidermis , Mesophyll cell, Cortical cells, Pith parenchyma	Medullary rays, Cortical parenchyma
2	Protein	$^{+}$ve	$^{+}$ve	Scattered cells of cortex, Mesophyll cells and Pith	Epidermis ,Cortical parenchyma, Pith parenchyma
3	Tannin	$^{+}$ve	$^{+}$ve	Scattered cells of mesophyll, Mid-rib pith parenchyma.	Vascular bundle and Scattered cells of medullary ray.
4	Saponin	$^{+}$ve	$^{+}$ve	Upper and lower epidermis and Mid-rib pith	Scattered cells of cortex and Medullary rays, and Pith parenchyma
5	Fat	$^{+}$ve	$^{+}$ve	epidermis in both surface palisade parenchyma, and phloem parenchyma	Scattered cells of pith ,Cortex, and Medullary rays
6	Glucoside	$^{-}$ve	$^{-}$ve	epidermis, collenchyma , parenchyma , sclerenchyma , secretory cavity next to the vascular bundle	periderm ,cortical Parenchyma , sclerenchyma , secretory cavity next to the vascular bundle
7	Alkaloids	$^{+}$ve	$^{+}$ve		
	a) Mayer's reagent	$^{+}$ve	$^{+}$ve	Upper epidermis Scattered cells of mesophyll, Mid rib pith parenchyma	Scattered cells of cortex, and Vascular bundle
	b)Wagner's reagent	$^{+}$ve	$^{+}$ve	Upper and lower cells of epidermis ,Pith parenchyma	Scattered cells of cortex, Medullary rays, Vascular bundle

Syzygium cumini

starch test

T.S. of leaf



T.S. of stem

Plate 1

Syzygium cumini

Protein test

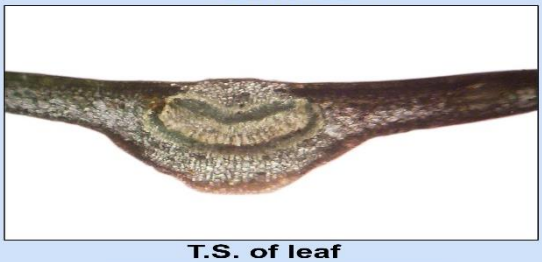
T.S. of leaf

T.S. of stem

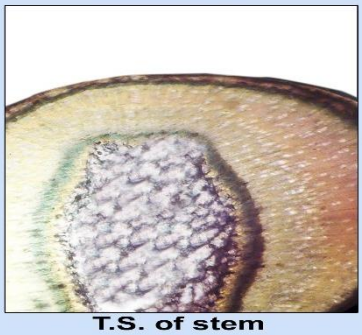
Plate 2

Syzygium cumini

Tannin test



T.S. of leaf

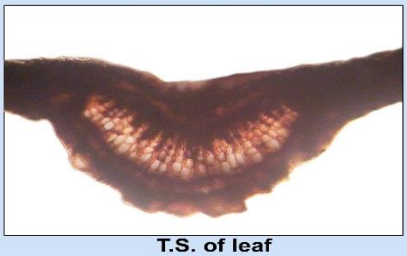


T.S. of stem

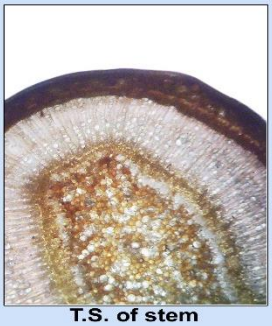
Plate 3

Syzygium cumini

Saponin test



T.S. of leaf



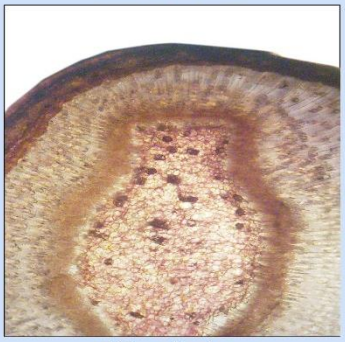
T.S. of stem

Plate 4

Photo Plates of Histochemical test of fresh section of leaves and stem of *Syzygium cumini* Linn

Syzygium cumini
Fat test

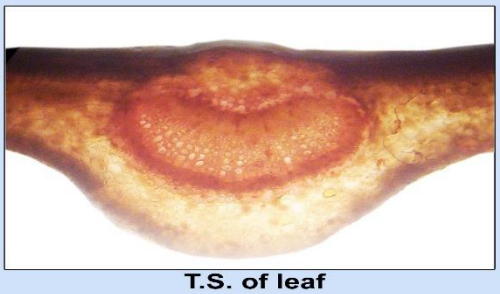
T.S. of leaf



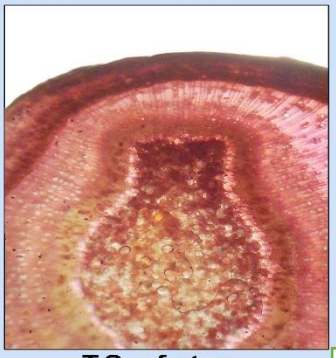
T.S. of stem

Plate 5

Syzygium cumini
Glucosides test



T.S. of leaf

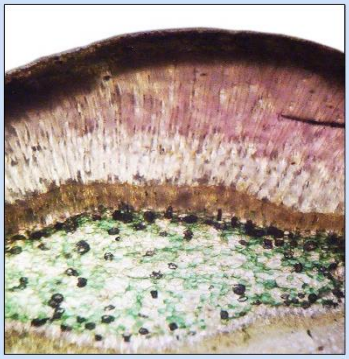


T.S. of stem

Plate 6

Syzygium cumini
Alkaloids test for Mayers reagent

T.S. of leaf



T.S. of stem

Plate 7

Syzygium cumini
Alkaloids test for wagners reagent

T.S. of leaf

T.S. of stem

Plate 8

In summary, through histochemical investigation of Syzygium cumini it possible to identify in tissue level, region of synthesis and storage of metabolites of pharmacological uses of leaves and Stem of Syzygium cumini. The active secondary metabolite presence in different location in leaf and Stem also. The potentially active compounds were concentrated on different tissues present primarily among the parenchyma and epidermal cells, which are differentiated from the others only by the presence of chemical compounds.

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