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RESEARCH ARTICLE

MICROMORPHOLOGICAL AND FTIR SPECTRAL ANALYSIS OF *SOLANUM TRILOBATUM* L. (SOLANACEAE) - A MEDICINALLY VALUED THORNY CREEPER FROM SOUTH INDIA

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Abstract

The ultrastructural features of the medicinal species of *Solanum* viz. *Solanum trilobatum* was investigated with Scanning Electron Microscopy. Pollen, trichomes and seed texture pattern were analyzed. The traits like pollen morphology and seed surface architecture were regarded as genetically determined traits and seldom influenced by ecological conditions. FourierTransforrm Infra Red spectral data was obtained using dried leaf tissue samples which was further analyzed with a view to distinguish the functional chemical groups in the pharmacobotanical herb which in turn provides relevant preliminary informations for chemotaxonomical perusal. The morphology of the spermoderm as well as Infra Red spectral peaks were found to be useful as taxonomic tools for species level identity as there is high degree of adulteration possibility due to increased demands.

Introduction:-

Solanum L. consists of over 2000 species distributed worldwide (Knapp, 1991) is the largest in Solanaceae and is one of the largest among flowering plants (Olmstead and Palmer, 1997). The species are a common source of vegetables (Omidiji, 1982), medicinal herbs (Caicedo and Schaal, 2004) and contain unique alkaloids and other biochemical constituents used for the treatment of diverse ailments (diabetes, cholera, bronchitis, high blood pressure) and as laxatives (Lester and Seck, 2004). *Solanum trilobatum* L. is a thorny creeper with bluish violet flowers and is widely distributed in Bengal, Uttar Padesh and Southern India. This important medicinal plant has been used in herbal medicine to treat various diseases like respiratory problems, bronchial asthma and tuberculosis (Chopra et al., 1958). This plant is well known in Ayurveda and Siddha systems. The roots, berries and flowers are used for cough (Swathy et al., 2010)]. The leaf extracts were used to increase male fertility and used to cure snake poison (Kumar et al., 2011). *Solanum trilobatum* possesses antioxidant, hepatoprotective, anti inflammatory and anti ulcerogenic properties.

Though the species is having immense medicinal potential, a taxonomic approach in an ultra structural perspective has not been attempted. Mostly, the species is harvested from field and marketed in dried forms and as in other pharmacobotanical herbs, the increased demands often leads to adulteration with similar looking taxa which cannot be easily distinguishable, especially in dried stage. The trichomes, spermoderm architectural patterns and pollen morphology characterize a species taxonomically, which in several instances resolves many systematic puzzles. The taxonomic value of epidermal morphology is well documented in botanical literature (Jayeola et al; 2001; Adedjii and Illoh, 2004). In comparative investigations of angiosperms, trichomes were considered to have great importance. In *Solanum*, seed morphology has been very useful in distinguishing both species groups and occasionally in distinguishing closely related species or species complexes (Gunn and Gaffney, 1974; Edmonds, 1983; Lester, 1991; Knapp and Helgason, 1997).

The main structure of taxonomic interest in the seed epidermis of the Solanaceae has been the anticlinal wall which, in several species, is naturally exposed to surface view by disintegration of the outer periclinal wall during seed development. The mature anticlinal walls and inner periclinal walls are unevenly thickened by secondary depositions imparting a pyramidal or triangular appearance in transverse section. Further uneven depositions near the outer periclinal walls produce appendages variously named as `hairs ', `spurious hairs ', `pseudohairs', ` hair-like structures ', ` scales ' and`zig-zag outgrowths' (Gunn and Gaffney,1974; Corner, 1976; Edmonds, 1983). The shape of the anticlinal wall and its projections have been found to be specific to species or sections of the genus (Whalen, 1979; Edmonds, 1983).

Morphological characteristics of pollen grains also can be useful characters in studies of plant taxonomy because many pollen traits are influenced by the strong selective forces involved in various reproductive processes, including pollination, dispersal, and germination (Erdtman 1952; Moore et al., 1991; Stuessy, 1990). However, the use of pollen morphology as a taxonomic character is challenging, and pollen characteristics must be considered in concert with other characteristics in evolutionary reconstructions.

Fourier transform infrared spectroscopy (FTIR) provides biochemical profiles containing overlapping signals from a majority of the compounds that are present when whole cells are analyzed. Leaf samples of higher plant species and varieties were subjected to FTIR to determine whether plants can be discriminated phylogenetically on the basis of biochemical profiles. The principal component analysis of Fourier Transform Infrared (FTIR) data confirmed most of morphological classifications of the species proposed in previous works.

Thus in the present ultra structural study of foliar trichomes, spermoderm sculpturing patterns, pollen morphology together with FTIR spectral pattern has been successfully employed in characterizing the highly valued and most exploited medicinal plant *Solanum trilobatum*.

MATERIALS AND METHODS

Plant materials

Fresh specimens of pollen, seed, and leaves of *Solanum trilobatum* were collected from Thiruvananthapuram district of Kerala, India, and were identified by comparison with the voucher specimen from Kerala Forest Research institute (KFRI, Kerala). The voucher specimens were deposited at the herbarium of JNTBGRI Palode.

Scanning electron microscopy (SEM)

Leaf

Fresh leaf pieces (10 **x** 10 mm^2) were immersed in a fixative solution of 3 % glutaraldehyde in 0.1 M phosphate buffer for 24 h. Samples were washed for 15-30 min with the buffer and dehydrated in graded ethanol series. Samples were then critical-point dried using $C0_2$, sputter coated with gold under vacuum and viewed with Zeiss EVO 18 i (S-450) scanning electron microscope operating at 15 kV. Images were captured digitally with an Image Slave computer programme for Windows.

Seeds

Mature dry seeds (without fixation) were washed thoroughly, dried and then glued to aluminum stubs and coated with gold-palladium to a thickness of 40 to 50 nm using a JEOL Finecoat Ion Sputter JFL 1100. The specimens were viewed in Zeiss scanning electron microscope (Zeiss EVO 18 i (S-450) and photographed at different magnifications. At least 15 seeds were randomly selected and studied.

Pollen grains

Anthers separated from 70% alcohol fixed flower buds are used for pollen collection. Acetolysis was carried out (Erdtman, 1960) and the acetolysed grains attached to stubs with double-faced carbon tape. The stubs are gold-coated in sputter coater for one minute, and examined under electron microscope (Zeiss EVO 18 i (S-450). About 75 grains were observed in different fields and photographs taken at different magnifications. The terminology used is in accordance with Punt et al., (2007).

FTIR spectroscopy

The leaves (approximately 3-4cm) were taken from different plants and were pooled as one sample. Then the samples were immediately dried in an oven for 2d at 60^oC. Tablets for FTIR spectroscopy were prepared in an agate mortars, by mixing leaves powder (2 mg) with KBr (1:100 p/p). The absorbance spectra were measured between 300 and 4500 cm^{-1}. At least three leaves were collected from each plant and at least three spectra were obtained. Monitoring of position of bands representing the functional groups was performed with Knowitall 7.8 software. The region between 3000 and 2800 cm-1 in the spectra was selected to analyze lipids, region between 1800 and 1500 cm-1 was selected to analyze proteins and the spectral region between 1200 and 1000 cm-1 was selected to analyze carbohydrates.

RESULTS AND DISCUSSION

Trichome morphology

Egandular trichomes were absent on either surface of the leaves. The leaves appeared to have a reflective cuticular layer on the upper surface. The lower surface of the leaves had few scattered glandular trichomes (Fig.1). Glandular trichomes were short, having short stalks and capitate heads (Fig. 2).

The trichomes exhibit a tremendous species specific diversity in shape (Payne, 1978) and hence often used as diagnostic characteristics for the identification of plant species (Reis et al., 2002). According to Wagner et al., (2004) , non glandular trichomes are present in most angiosperms but could be seen in some gymnosperms and bryophytes. In contrast, Fahn (2000) reported that glandular trichomes are usually multicellular and found to occur in approximately 30 percentages of vascular plants. . They have the capacity to produce, store and secrete large amounts of different classes of secondary metabolites.

Leaf trichomes are described as traits related to both water control and resistance against herbivory in several plant species (Molina-Montenegro et al., 2006). Trichomes constitute a mechanical barrier that hinders insect movement and/or feeding (Baur et al., 1991), and chemical compounds in glandular trichomes can be deterrent or toxic to several herbivores (Buta et al., 1993). Glandular trichomes are characterized by having " heads " (Glands) that release, on contact,sticky and/ or toxic exudates that may entrap, irritate or potentially kill some pests (Simmons et al., 2003). These glands contain important secondary metabolites including terpenes, essential oils, flavonoids and lipophilic components (Ascensao et al., 1999). In most species the source of these secondary metabolites has been attributed to the trichomes (Buta et al., 1993). The possession of glandular trichomes is characteristic of the genus *Solanum* and of many other members of Solanaceae with the exception of *Nicotiana glauca* and *Solandra nitida* (Maiti et al., 2002).The foliar ultramorphology of the studied species thus gives informations regarding its adaptation to environmental stresses apart from their role in taxonomic realm.

Seed surface architecture

The seeds reniform in shape, having wing like distensions which make them somewhat larger (Fig.3). The surface displays a reticulate pattern with fibrils (Fig. 4) and the hilum is basal in position (Fig. 5). The cells were polygonal to subrounded in shape, isodiametric. The lateral testal cell walls sparsely papillate.

Seed morphology has been shown to provide useful characters for the analysis of taxonomic relationships in a wide variety of plant families (Barthlott, 1981, 1984; Shetler, 1986; Takhtajan, 1991). In addition to gross morphology of seeds, the details of the sculpturing of the outer seed coat can be quite variable and of systematic importance. Recently, the systematic implications of seed coat diversity has been carried out in *Ipomoea* (Abdel khalik, 2013), *Minuartia* (Mostafavi et al., 2013) and *Lepidium* (Bona, 2013). The seeds of *Solanum trilobatum* are reniform in shape having a length × width measurement of 3.5/ 3.48 × 2.54/ 2.52 mm. The surface is roughly reticulate and the fibrillar structures from the distal ends project out and merge laterally. The cell lumens are shallow and the lateral testal cell walls are sparsely papillate. All these features are of particular systematic relevance that delineates the species. In other taxa also, the spermodem sculpturing patterns have been found to be different in different species of the genus and hence of taxonomic importance (Mostafavi et al., 2013). Considerable differences were also observed in the seed coat morphology both among and within the genera in the tribe Hyoscyameae of Solanaceae (Zhi Yun Zhang et al., 2005).

Pollen grains

Grains globose, triangular obtuse convex, 22–22.9 × 21–21.8 μm in polar view (Fig. 6), trizonocolporate, operculum prominent and globular (Fig.7); Colpus is multi-bridged (Fig.8) and aspis is absent; Exine ornamentation is granulate (Fig.9).

Pollen characters have received more attention in taxonomy and pollen morphology. However, the ultra-structure of the pollen wall, its stratification and internal structure can hardly be studied by light microscope (Zavada, 1990). Therefore Scanning and Transmission Electron microscopy become necessary in examining these characters, El-Ghazaly (1990) and Harky, et al., (2000) reported on the morphology of pollen grains of many plant species. *Solanum trilobatum* has grains that are globose, triangular obtuse convex, 22–22.9 × 21–21.8 μm in polar view, trizonocolporate, operculum prominent and globular. The granulose exine ornamentation characterizes wild germplasm. The colpi region showed a tendency for multibridging which can be considered as a step towards additional protection. Opercula are rare in basal angiosperms with monosulcate pollen. Among monocots, opercula are characteristic of the orders Asparagales, Liliales and Poales that might have undergone evolution due to selection pressures. Opercula protect the pollen grains against desiccation and pathogen invasion. Recently pollen morphology of selected taxa of *Solanum* has been carried out by Anil kumar et al., (2015) and the grains of *Solanum* *trilobatum* are described as coming under wild germplasm owing to the presence of prominent opercula, granulose exine as well as multibridging, all of which are considered to impart additional protection to the pollen germplasm.

FTIR spectrum
IR peak from 513 cm-1 to 518 cm-1 is exhibited. Peak at 3869 cm-1 is characteristic (Fig. 10).

Chemotaxonomy has strongly influenced the entire field of biology, which is also useful for plant systematics. Fourier Transform Infrared (FTIR) Spectroscopy is a rapid, noninvasive, high-resolution analytical tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". This technology allows detecting the whole range of infrared spectrum in measurements of biological specimens (Griffiths and de Haseth, 1986). Thus, these "fingerprints" are made up of the vibrational features of all the cell components, i.e., DNA, RNA, proteins, and membrane and cell-wall components. The characteristic peak at 3869 cm-1, shown by *Solanum trilobatum* represents the occurrence of alkenes and aromatics with C-H stretch- alkynes, carboxylic acids, primary and secondary amines and amides, alcohols and phenols and many of these compounds might be contributing to the proven pharmacognostic potentials of the species and thus the IR fingerprint can be exploited as a tool for accurate identification of the taxa, especially when it is obtained in dried forms as in local markets. The IR fingerprinting of *Solanum* species from the state of Kerala has been reported earlier in which a comparison of the IR finger prints of various species has been provided (Anil kumar and Murugan, 2012). In plant classification, Kim et al., (2004) have proposed this approach as robust in chemotaxonomic classification of flowering plants, and previously this method was used to identify the species in *Hypericum* L. and *Triadenum* Raf. (Lu et al., 2004).

Figures:-
Fig. 1 - *Solanum trilobatum* L., lower surface of leaf showing scattered glandular trichomes
Fig. 2 -Glandular trichome showing capitates head
Fig. 3 -Entire seed showing reniforrm shape
Fig. 4 - Seed surface showing retticulate architecture
Fig. 5 - Seed- hilum region
Fig. 6 - Pollen grain, polar view
Fig. 7 - Pollen grain, equatorial view
Fig. 8 - Colpus region of pollen grain showing multibridging tendency
Fig. 9 -Ornamentation in the apocolpium region
Fig. 10 -IR spectrum

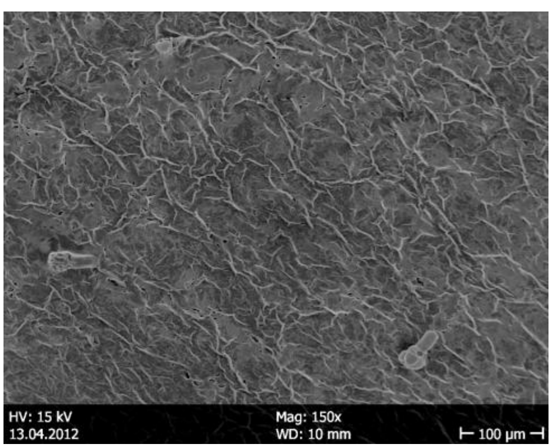
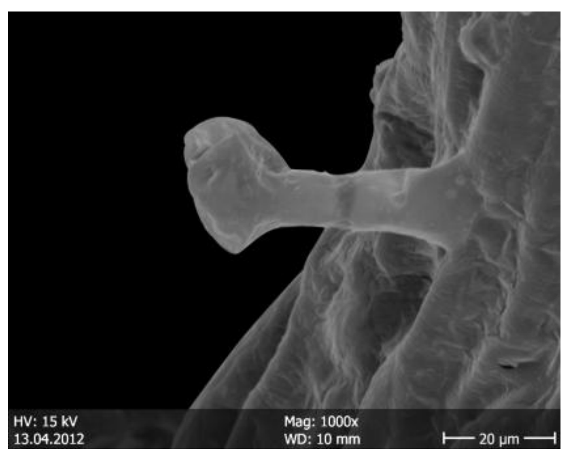


Fig. 1



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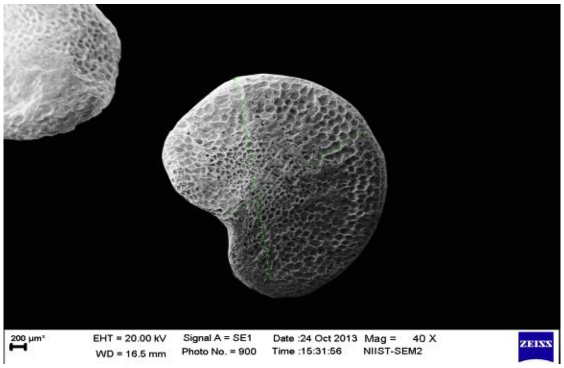


Fig. 3

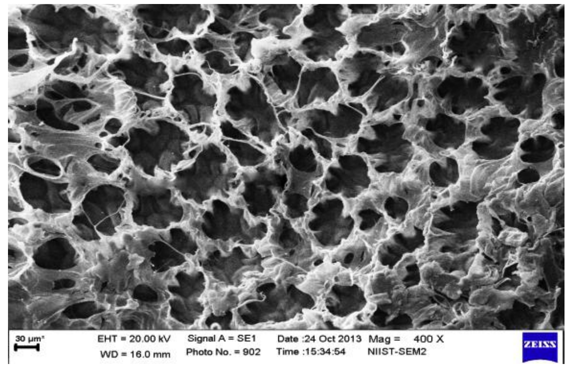
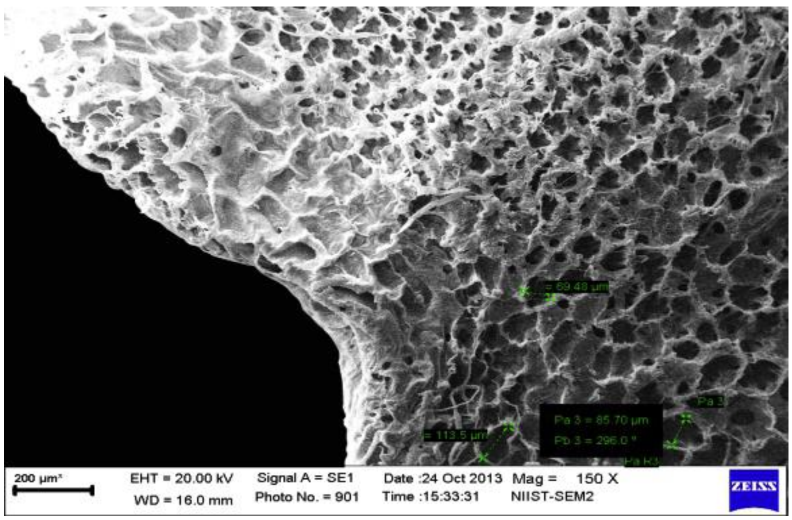


Fig. 4



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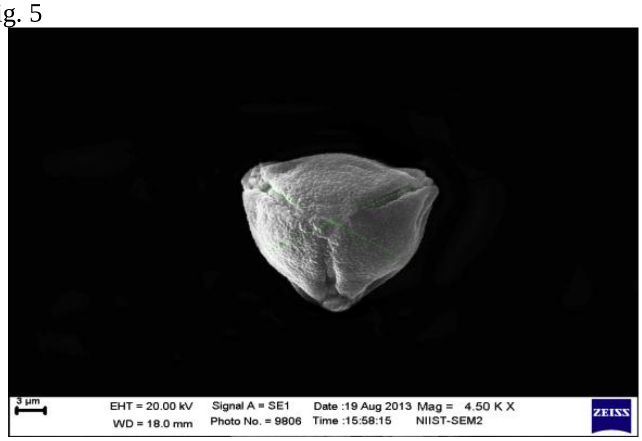

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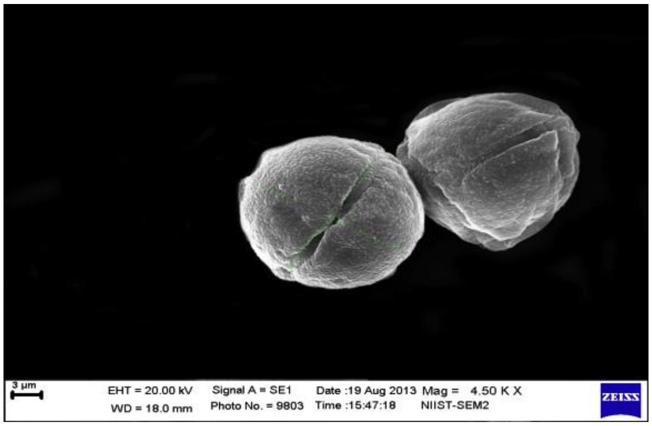

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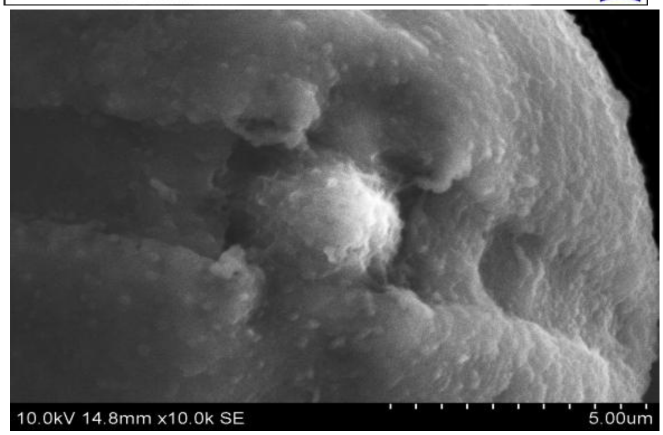

Fig.8

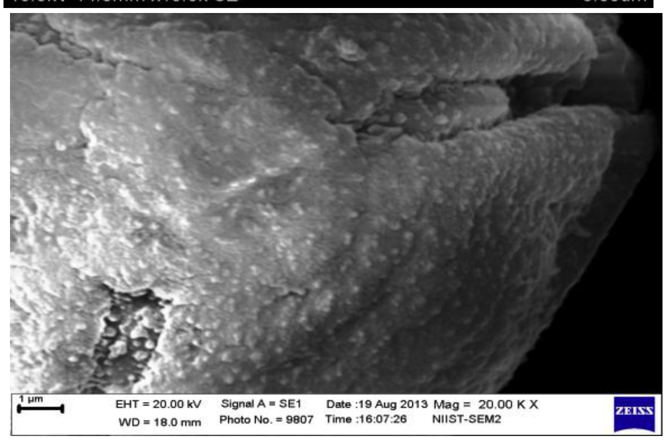

Fig. 9

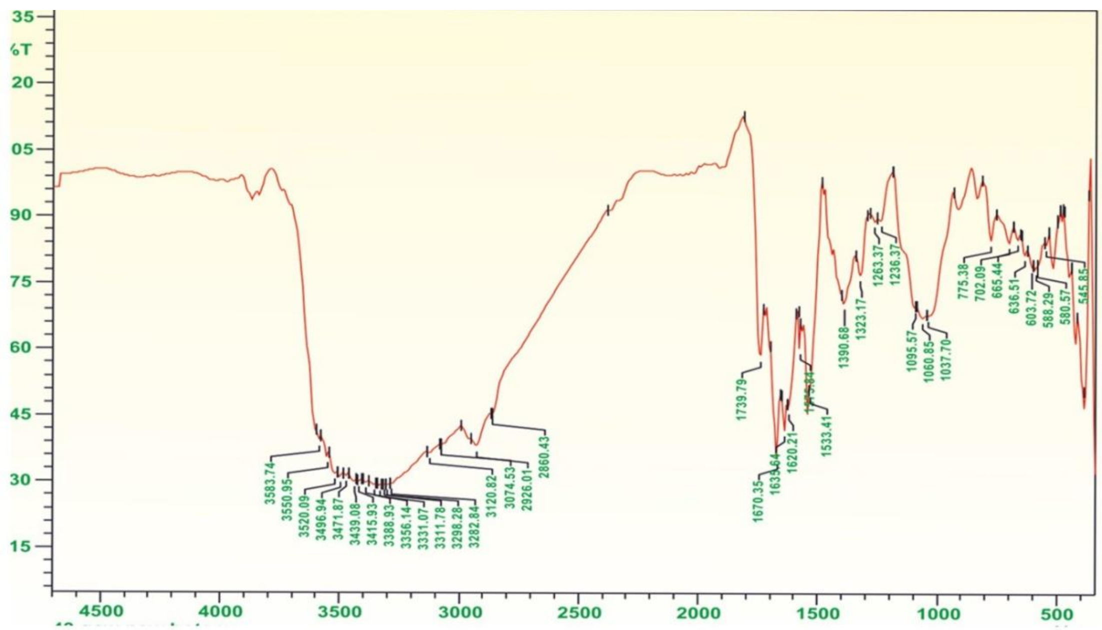

Fig. 10.

Conclusion:-

Thus it is evident that the selected species of *Solanum* shows distinct morphology with respect to trichomes, spermoderm pattern as well as pollen grains and these features can be effectively employed as taxonomic tools for its identity. Even though different *Solanum* species do differ in their gross morphology, there is still taxonomic ambiguity in several species worldwide. In this scenario, this kind of work can reflect the exact taxonomic placement of various species as all these traits are proved to be genetically controlled in the plants. The FTIR spectral data was also found to be useful in determining the phytochemically relevant functional groups so that the potential of the plant species can further be exploited using other techniques. It can also provide the characteristic fingerprint of the sample even from dried tissues. Further studies are warranted employing the molecular tools with DNA, RNA and proteins which can discriminate the plant species at the genetic level and can provide data regarding their genetic and phylogenic affinities and interrelationships.

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