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S. Chinnammal
Department Of Botany,
Kunthavai Naacchiyaar Govt.Arts College For Women
(Autonomous)
Thanjavur – 613 007,
Tamilnadu

G. Subasri *
Assistant professor, PG and Research
Department Of Botany,
Kunthavai Naacchiyaar Govt.Arts College For Women
(Autonomous)
Thanjavur – 613 007,
Tamilnadu

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PHARMACOGNOSTICAL AND PHYTOCHEMICAL INVESTIGATION IN Scoparia dulcis L. LEAVES

S. Chinnammal and G. Subasri*,

ABSTRACT

In the present study was to investigate the pharmacognostical phytochemical screening, histochemical and Fluorescence of *S. dulcis* leaves extract. Physicochemical analysis of *S. dulcis* leaves powder was investigated. This work established the description loss on moister content, total ash, alcohol soluble extractive and water soluble extractive of *S. dulcis* leaves were found to be 7.00%, 6.5%, 9.31% and 10.37% respectively. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvent. he phytochemical screening *S. dulcis* leaves showed that the presence of tannin, saponins, flavonoids, glycosides, terpenoids, steroids, polyphenol, alkaloids, triterpenoids, carbohydrate and anthroquinones whereas phlobatannin was absent in methanol and aqueous extracts. Protein were present only aqueous extract. The histochemical analysis further confirmed in the presence of flavonoids, alkaloids, glycosides, terpenoids, steroids, polyphenol, tannin and saponin. The fluorescence behavior of *Scoparia dulcis* L. leaves powder proved by this study

Keywords: Phytochemical, Antimicrobial, *Scoparia dulcis* L.

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INTRODUCTION

Pharmacognosy, the modern science of natural medicines, is based on traditional medicines used in different parts of the world. Traditional medical heritages of Ayurveda, Traditional Chinese medicine, Greco-European medicine, Egyptian medicine, Kampo medicine and others are important precursors for the development of Pharmacognosy and pharma science. European legendary scholars gradually compiled traditional medicinal knowledge and pharmacy practices in the form of Materia Medica that eventually evolved into a new stream of learning, i.e. Pharmacognosy (Balunas and Kinghorn, 2005). The increasing use of modern medicines during the latter half of the 19th century and early days of 20th century gradually combined the contemporary developments in Pharmacognosy and clinicalaspects of pharmacy education became dominant. However, during the latter half of 20th century the remnants of conventional Pharmacognosy re-emerged along with the herbal resurgence in the western world and eventually quality, safety and efficacy of natural remedies became general issues. However a huge proportion of Amazon, the Mediterranean and most of the tropical biomes are still unexplored. Therefore, urgent action is essential for bioprospecting of natural resources and conservation of indigenous healing knowledge and technologies in these areas (Blondeau et al., 2010). Plant and plant products play a wide range of biological properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. Keeping in view, the present study to investigate the phytochemical analysis of *Scoparia dulcis* L. leaves.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Scoparia dulcis* L. were collected in December 2017 from Manjappattai, Pudukkottai, Tamil Nadu, India. The *S. dulcis* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Leaves was spread out in a plain paper and shade dried at room temperature for about 10 days and makes a fine powder using grinder mixture. The powder materials were used for further studies.

Pharmacognostic study

Chemicals and Reagents

All the chemicals and reagents like chloral hydrate, phloroglucinol, acetic acid, Chloroform, Ethyl acetate, Ethanol, used were of analytical grade.

Procedure:

The leaf of *S. dulcis* was collected and rotatory microtome sections were taken for leaf to obtain a thin section. The thickness of the section was 10-12 micrometers. Anatomical study invariably slides were prepared. The transverse sections of required parts (leaf) was taken on a glass slide to which are added a few drops of chloral hydrate and was heated for 1-2 min, After placing a cover slip ,care should be taken to avoid air bubbles and to see that there is sufficient chloral hydrate under the cover slip . Excess of chloral hydrate outside the cover slip is to be withdrawn using a blotting paper (Chloral hydrate is used to clear the tissues and to bring in clarity of the view) Lignified tissue are to be confirmed by staining .To the powder a few drops of mixture of 1:1 Phloroglucinol +Conc HCl was added and after 3 to 4 minutes observed under microscope. The well-known identifying characters were taken Photomicrographs by Sony digital camera under microscope (10 x & 40x) (Wallis, 1989; Dutta, 1971).

Organoleptic character

The organoleptic investigations (color, shape, odour and taste) were performed.

Determination of physicochemical parameters

Physicochemical parameters of the powdered *S. dulcis* leaves such as ash value, extractive value, loss on drying and crude fiber content were performed according to the method described in WHO guidelines (WHO, 1998).

Preparation of plant extract:

2 gram of the powder of *S. dulcis* leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing *S. dulcis* leaves were shake it well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper No.1 and filtrate used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984). Determination of Fluorescence behavior (Rao *et al.*, 2011)

Histochemical tests

A cross section leaves sample was placed on a grease free microscopic slide and treated with specific chemicals and reagents and waited for 1-2 minutes. A positive test for histochemicals was indicated by the appearance of the appropriate colour change after application of the reagent. Using a light microscope to observe and record any colour changes. The leaf cross section treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids. Leaf cross section treated with ferric chloride to give Dark blue to black indicates the presence of tannin. Leaf cross section treated with H_2SO_4 (few drops) to give yellow colour indicates the presence of Saponin.

RESULTS AND DISCUSSION

Anatomical characteristics

Transversal section of the leaf

Pharmacognosy may be defined as *"*an applied science that deals with the biologic, biochemical and economic feature of natural drugs and their constituents.*"* Modern aspects of science include not only the crude drugs but also their natural derivatives. Plant anatomy, in turn, has given rise to the independent science of cytology, which is the study of the cell, a rapidly developing field that plays a great role in the understanding of vital processes in general and of the phenomena of heredity and mutability in particular. Plant Anatomy is the branch of botany concerned with the internal structure of plants. It is closely related to plant physiology, the science of the vital processes which take place in plants.

Transverse section of *Scoparia dulcis* L leaf through the midrib showed an upper and lower, single-layered epidermis that was externally covered with a thick, striated cuticle, a few epidermal cells on both lower and upper surfaces, parenchymatous cells that were thin-walled and isodiametric to circular (Plate 2**)**. Intracellular spaces were present in ground tissue and the stele was crescent-shaped and composed of bicollateral and open vascular bundles. The xylem consisted mostly of vessels and tracheids, and a strip of cambium was present between the xylem and phloem tissues. The lamina which was dorsiventral with the mesophyll, was seen to be differentiated into a palisade and spongy tissue. The upper and lower epidermises were covered externally with a thick, striated cuticle. Below the upper epidermis were three rows of elongated, closely arranged, palisade parenchyma. Spongy parenchyma tissues were almost radially elongated with intracellular spaces. Central cells were irregular in shape; laticifers and vascular bundles were also present scattered in this region.

Dinesh Kumar *et al* (2014) examined the pharmacognostic evaluation of *Clerodendrum phlomidis* Linn. in terms of organoleptic, fluorescence analysis, macro-microscopy and physicochemical parameters. The characteristic macroscopic study showed that the root consists of 7-15 cm long, 0.2 -3.0 cm thick pieces which are cylindrical, tough and yellowish-brown externally, with hard fracture and slightly astringent taste. The main microscopic characters of the root show exfoliating cork, having 10-15 rows of tangentially elongated, thick-walled cells.

Organoleptic characters

Organoleptic evaluation can be done by means of organs of sense which includes the sensory parameters and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity and degree of purity. The organoleptic investigations (color, shape, odour and tastee) were performed. The results of organoleptic characters such as colour, taste, odour, texture and size are shown in Table 1.

Physicochemical analysis of *Scoparia dulcis* L

The results of physicochemical parameters such as moister content, total ash, alcohol soluble extractive and water soluble extractive are shown in Table 2.

According to WHO (1992, 1996a and b) standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion.The parameters which are studied moisture content, description, loss on drying, total ash, acid-insoluble ash, sulphated ash, alcohol soluble and water-soluble extractive values, etc.

The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. The standardization of crude drugs is important before any work carried out. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. The physicochemical test is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken (Tatiya *et al.*, 2012).

Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not (Tatiya *et al.*, 2012).

Kaskoos and Ahamad (2014) reported that the quality control parameters like extractive of

Andrographis paniculata with different solvents, ash values, foreign organic matter and loss on drying were determined. Ujwala, (2013) reported the physicochemical analysis of the extract of *Bixa orellana.*

Similarly work was done by Rajmohanan *et al.* (2014) the physicochemical studies on the *Cayratia pedata* (Lam) (Juss ex)Gagnep. Leaves showed significant total ash (9.62 – 10.50 %), acid-insoluble ash (3.2 – 3.9 %), loss on drying at 105 °C (7.23 – 8.25 %) and water soluble extractive (2.4 – 3.4 %) alcohol soluble extractive (8.95 – 9.35 %) respectively. Ash value used to determine quality and purity of crude drug.

The earlier studies of Giby Abraham (2015) showed that the physicochemical analysis of *Vernonia cinerea* L. Physicochemical such as total moisture content (4.6 %), total ash (7.44 %), water soluable ash (4.57 %), acid insoluble ash (0.29 %), ethanol extractive (5.29 %) and water extractive (10.24 %) .

Phytochemicals analysis

In the present study was carried out on the *Scoparia dulcis* L leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *S. dulcis* leaves investigated and summarized in Table-3 and Plate 3 and 4. The phytochemical screening *S. dulcis* leaves showed that the presence of tannin, saponins, flavonoids, glycosides, terpenoids, steroids, polyphenol, alkaloids, triterpenoids, carbohydrate and anthroquinones whereas phlobatannin was absent in methanol and aqueous extracts. Protein were present only aqueous extract.

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils, minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites (Sofowara, 1993). Leo Stanley *et al.* (2011) reported that leaves of *C. pedata* showed the presence of alkaloids, carbohydrates, steroids, tannin, phenolic compounds, flavonoids and terpenoids. Dinesh kumar *et al.* (2011) has been reported to terpenoids, flavonoids and tannin are present in *C. trifolia.* Rajmohanan *et al.* (2014) investigated the preliminary phytochemical analysis of various extracts of leaves of *C. pedata* and showed the presence of carbohydrates, flavonoids, tannins and phenolic compounds and terpenes.

Abuzar *et al.* (2013) reported the phytochemical analyses of *Heliotropiumdasycarpum*.L were evaluating the presence of secondary metabolites in drug sample. The results showed the presence of alkaloids and cardiac glycosides while the saponins, anthroquinone, glycoside and tannins were absent in the plant extract.

Alkaloids such as solasodium have been indicated as a starting material in the manufacture of steroidal drug. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects. They exhibited marked physicological activity when administered to animals (Okwu, 2004). Alkaloids have established broad spectrum antibacterial activity and are also used as analgesics and narcotics for pain relief. Alkaloids are very important in medicine and constitute most of the valuable drug. They have marked physicological effect in animals (Edeoga *et al.*, 2006).

Tannins are phenolic phytochemicals, which are natural constituents of green tea, are considered to have cancer preventive properties (Lambert and Yang, 2003; Niraimathi *et al.*, 2012). Condensed tannins, isolated from black beans, did not affect the growth of normal cells, but induced cell death in cancer cells in a dose dependent manner (Awika *et al.*, 2004). Studies in animal models and with cultured human malignant cell lines have demonstrated both the antitumor and cancer preventive activities of methanolic extract of *Psidium guajava* leaves and its main ingredients. It was suggested that these effects of methanolic extract might be due to their content of flavonoids, tannins, alkaloids and saponins reported earlier (Vikrant Arya *et al.*, 2012).

Histochemical analysis of leaves powder of *Scoparia dulcis* L. leaves

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. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major phytocompounds (Krishnan *et al.*, 2001). In the present study, *S. dulcis* leaves were treated with specific chemicals and reagents. The *S. dulcis* leaves cross section treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids, treated with $FeCl_3$ gave green colour indicates tannin and treated with concentrated H_2SO_4 gave yellow colour indicates saponin ((Table 4 and Plate 5**)**. This results further confirmed the presence of phytochemicals.

John Peter Paul, (2014) attempt was taken for histochemical and fluorescence analysis of *Turbinaria ornata* (Turner). Histochemical analyses of the plant were carried out using light microscopy and fluorescence study was analyzed by UV lamp. Results of histochemical tests showed positive reaction to phenol compounds, polyphenol and tannin in the thallus. Fine powder and different solvent extracts of *Turbinaria ornata* obtained using petroleum ether, benzene, chloroform, acetone, ethanol and aqueous were examined under visible and UV light.

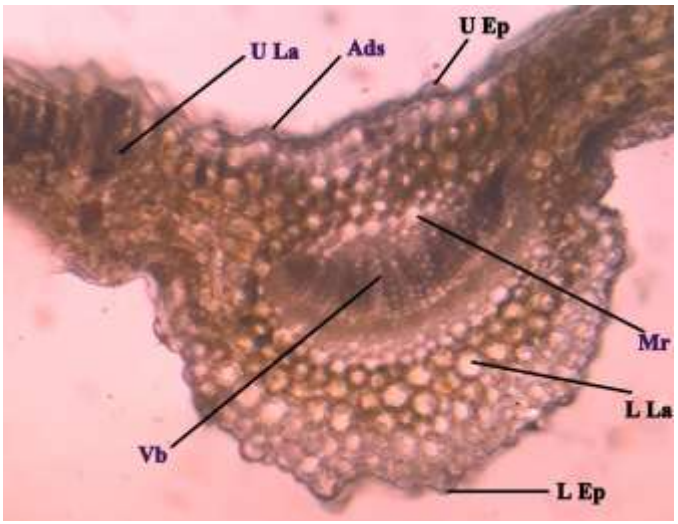
Fluorescence behavior of *Scoparia dulcis* L. leaves powder

Fluorescence analysis of entire leaves of *S. dulcis* leaves has been carried out in daylight and under UV light. Fluorescence analysis of leaf powder of *S. dulcis* leaves was carried out by the treatment of different chemical reagents such as H_2SO_4, HCl, NH_3, HNO_3, CH_3OH and NaOH. The powders were observed in normal daylight and under short (245 nm) and long UV light (365 nm) and the results were presented in Table 5 and Plate 6.

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many products, which do not visibly fluorescence in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. (Tyler *et al.*, 1976).

In the fluorescence analysis, the plant parts or crude drugs may be examined as such or in their powdered form or in solution or as extracts. Although, in most of the cases the actual substances responsible for the fluorescence properties has not been identified, the merits of simplicity and rapidity of the process makes it a valuable analytical tool in the identification of plant samples and crude drugs (Denston, 1946). Hence, some drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (Kokashi *et al.*, 1958).

Plate 2 Transverse section of *Scoparia dulcis* L. leaf



Ads–Adaxial side, U Ep– Upper Epitermis, L Ep – Lower Epitermis, L La – Lower lamina, Mr – Midrib, U La – Upper lamina, Vb –Vascular bundle

Table 1 Organoleptic characters of *Scoparia dulcis* L leaves

S.No	Organoleptic characters	Result
1	Colour	Green
2	Taste	Sweet
3	Odour	Pungent
4	Texture	Smooth
5	Size	1-2 cm

Table 2 Physicochemical analysis of *Scoparia dulcis* L

S.No	Tests	As per analysis
1	Moister content	7.00%
2	Total Ash	6.5%
3	Alcohol soluble extractive	9.31%
4	Water soluble extractive	10.37%

Table.3 Qualitative analysis of Phytochemicals in *Scoparia dulcis* L. leaves

S.No	Test analysis	Methanol extract	Aqueous extract
1	Tannin	+	+
2	Phlobatannin	-	-
3	Saponin	+	+
4	Flavanoids	+	+
5	Steroids	+	+
6	Terpenoids	+	++
7	Triterpnoids	+	+
8	Alkaloid	++	+
9	Carbohydrate	+	+
10	Protein	-	+
11	Anthroquinone	+	+
12	Polyphenol	+	+
13	Glycoside	+	+

(-) Indicates Absence; (+) Indicates Presence; (++) highly presence

Table 4 Histochemical analysis of leaves powder of *Scoparia dulcis* L. leaves

S.No	Charecterisation	Observation	Result
1	Tannin	Green	+
2	Flavonoids	Yellow	+
3	Saponin	Yellow	+
4	Steroids	Green	+
5	Terpenoids	Orange	+
6	Alkaloid	Reddish brown	+
7	Glycoside	Brown	+
8	Polyphenol	Blue green	+

Note: (+) Presence;

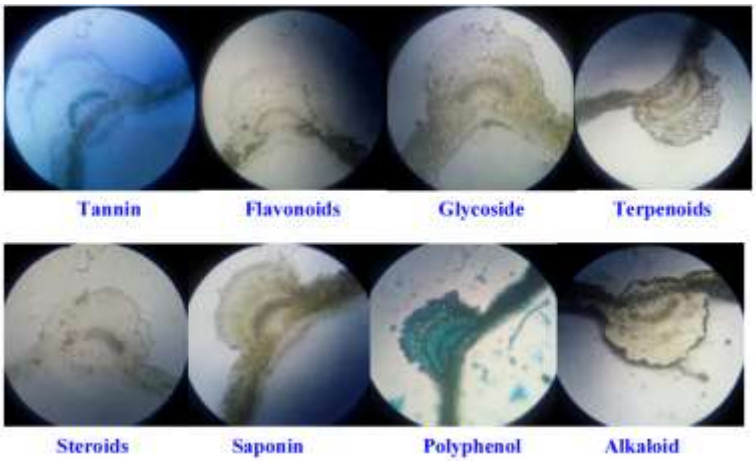


Plate 5: Histochemical analysis of *Scoparia dulcis* L. leaves cross section

Table 5 Fluorescence behavior of *Scoparia dulcis* L. leaves powder

S.No	Test	Visible Light	Short UV	Long UV
1	Plant powder	Green	Green	Black
2	Plant powder treated with water	Green	Green	Black
3	Plant powder treated with Hexane	Green	Green	Black
4	Plant powder treated with Chloroform	Green	Green	Black
5	Plant powder treated with Methanol	Green	Green	Black
6	Plant powder treated with Acetone	Green	Green	Black
7	Plant powder treated with 1N NaOH (water)	Green	Green	Black
8	Plant powder treated with 1N HCl	Brown	Green	Black
9	Plant powder treated with sulphuric acid with an equal amount of water	Green	Green	Black
10	Plant powder treated with Nitric acid dilute with an equal amount of water	Yellowish brown	Yellowish green	Black

CONCLUSION

Overall, it can be concluded from the present study that *S. dulcis* leaves contains rich source of phytochemicals. This study is the first scientific report that provides convincing phytochemicals evidence for the relevance of *S. dulcis* leaves thus providing scientific validity to its traditional consumption by the local populace of south India.

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