Pharmacognostical studies on the fruit of *Elaeocarpus oblongus* Gaertn.

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ABSTRACT

Elaeocarpus tectorius (Lour.) Poir, Synonym: *Elaeocarpus oblongus* auct. non Gaertn. Elaeocarpaceae, is a tree, found throughout Western Ghats, South India. The present study indented to establish the pharmacognostical and physicochemical quality control parameters of *E.oblongus* fruits to avoid confusion in taxonomic identification. Physicochemical evaluation of fruit was carried out according to the guidelines of WHO/QCMMP and Indian Ayurvedic Pharmacopoeia. The elemental analysis was done by using Perkin Elmer 5000 an atomic absorption spectrophotometer. Non glandular unicellular trichomes found to be distinguished character of powder analysis. It was quantified to be 700μm long and 400μm thick at the base. Lerachysclereids were found plenty in powder. The rosettes type of calcium oxalate crystals were 15 μm in diameter. Cells of the endosperm showing darkly stained globular bodies and the cotyledon is 170μm thick. Physio-chemical parameters such as total ash and acid-insoluble ash (2.66% w/w, 0.66% w/w, respectively), extractive values (aqueous 31.068% w/w and alcoholic 30.94% w/w), foreign organic matter (0.5% w/w) and loss on drying (12% w/w) were estimated. Qualitative analysis showed the presence of Fructose, Glucose, Flavanoids and Tannins and Sterols and Phenolic compounds and fatty acids in the fruit. The quantity of elements (μg/g) in the fruit pulp powder was estimated by elemental analysis. The result shows Mn-53.5 and Zn – 46.2 were the major contents. While Pd- 14.3, Cu- 7.5 and Cr- 4.9 were minor contents. This study provided the pharmacognostical profile used to differentiate the other similar looking fruit from other ones of this genus.

Keywords: Western Ghats, Budagas, Ooty, Quality control, Elaeocarpaceae, Edible fruit

INTRODUCTION

Elaeocarpus is a genus of tropical and subtropical trees distributed from Madagascar in the west through India, Southeast Asia, Malaysia, Japan, and Australia to New Zealand, Fiji and Hawaii in the east with its estimated about 350 species.[1] In India most of the species grow in the Himalayan region.[2] *Elaeocarpus* species possess many biologically active molecules they are Indolizilidine alkaloids, Triterpenes, Tannin such as Geraniin and 3, 4, 5-trimethoxy geraniin, Grandisines, Rudrakine and Flavonoids; Quercitin. Most of the *Elaeocarpus* species have exhibited Anti-inflammatory, Antimicrobial, Anti anxiety and Analgesic, Antidepressant and Antihypertensive activities.[3] The ancient literature shows *Elaeocarpus* was used as an Ayurvedic medicine, but biological studies revealed that a number of other therapeutic uses of this genus make known its species as the chief source of multi-use medicinal agent proved in experimental animals.[4]

Elaeocarpus tectorius (Lour.) Poir, Synonym: *Elaeocarpus oblongus* auct. non Gaertn., Elaeocarpaceae,[5] is found wild in Western Ghats ascending to 6,000 ft.[6] The tribe of Nilgiris district todas, kurumbas, kothas, irulas, kattanaikes are broadly using these fruits for therapeutic purpose. During our field trip, we had seen that the fruits were very cheapest resource for the treatment of rheumatism and body pain. It has been reported further various

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ancient tribes were using this fruit for the treatment of leprosy, pneumonia, rheumatism, ulcers, piles, and dropsy.[7–9] Literature shows very few biological studies had been conducted on leaf, stem and stem bark. But there was no report on pharmacognostic evaluation of much used part of the tree the fruit. Since the fruits are edible and important seasonal food commodity (May to August) and it is extensively sold in the local market of Nilgiris district, Tamilnadu state, India. The current study, has decided to investigate the anatomical, physicochemical characters and the presence of sugars in edible fruits of *Elaeocarpus oblongus*. This is the first time the fruit was assessed for anatomical and physicochemical characters.

MATERIALS AND METHODS

Study area

Udhagamandalam commonly known as Ooty, is the administrative capital of Nilgiris district. The district, as the Blue Mountain is a tourist resort and populated by the tribes of Badagas and Todas, Irulas, Panias, Kurumbas. Ooty is spread out at 11.24 degree North latitude and 76.41 degrees of longitude.[10] The queen of hill-Nilgiris[11] is made the south boundary of the Western Ghats structure that extended continuously from Mumbai in the north to the Nilgiris in the south. The altitude of this region ranges from 1700 to 2400 m above the sea level. The annual average rainfall is 1590 mm.The annual temperature ranges from 4°C to 24°C.The monsoon starts from June to October. The lateritic, dark brown, loamy soil is present in the study area.[12]

Collection of specimens

The fresh fruits and plant specimen were collected early morning during the summer season in the month of May 2012 from the "Bikki" tree at the Edakadu village near to the Ooty hill station, South India. The plant material was taxonomically identified by Prof.P. Jayaraman, Taxonomist, Plant Anatomy Research Centre, Chennai. The Voucher specimen (No.PARC/2010/2098) was deposited in the medicinal plant documentation unit in Pharmacognosy and Phyto chemistry department, Nehru College of Pharmacy, Pampady, Thiruvilwamala-680597, Thrissur district, Kerala state, India. Fresh fruits were used for microscopic characterization while dried fruit pulp powder has been used to determine the physicochemical parameters such as ash values and extractive values and powder microscopic characters and qualitative and fluorescence analysis. Reagents, Chemicals of analytical grade were used from Sigma Chemical Co, St. Louis, USA and Fine Chemicals Ltd., Mumbai, India.

Microscopic slide preparation

Fruit was fixed in FAA (Formalin-5 ml + Acetic acid-5 ml + 70% Alcohol-90 ml). Later on microscopic slides were prepared according to the usual plant anatomy protocols.[13-14] The transverse cross sections of the fruit were obtained by cutting the paraffin embedded[15-16] specimen by Rotary Microtome. The thickness of the sections was measured about 10–12 µm. De waxing of the sections was done by standard procedure,[14] subsequently the sections were stained with toluidine blue.[17] Sections of the fruit were cleared in 30% sodium hypochlorite solution and stained with safranin.[14] Microscopic characterization of fruit was done by Olympus optical microscope attached to a digital camera Sony. The powder microscopy was done by standard procedures [18,19] and micro chemical tests for histological region were performed according to the standard methods.[15&20–22] An anatomical feature of the description was made with the help of the standard anatomy book.[23]

Behavior of powder with different chemical reagents

The powder material was treated with different chemical reagents to detect the phytoconstituents by observing colour changes under ordinary daylight and also the colour and consistency of extracts were also observed by the standard method.[24]

Fluorescence analysis of powder and extracts

The fruit extracts were examined and analysed in daylight, short and long UV light for fluorescence, according to the standard methods.[25]

Estimation of inorganic constituents

To estimate the inorganic elements contents, 1 g of the fruit pulp, dried powder was digested with concentrated nitric acid and perchloric acid (3:1) until a clear solution was obtained. Subsequent to cooling, the solution was made up to a specific volume with the de mineralized water and analysed in Perkin Elmer 5000 an atomic absorption spectrophotometer.[26]

Physicochemical parameters

Loss on drying,[27] pH of 1% aqueous soluble portion of the fruit was calculated at 25°C,[28] Ash values and extractive values were carried out based on the standard protocol.[19] Thin Layer Chromatography (TLC) was performed for fruit extracts according to the standard procedure.[29,30]

Determination of fruit sugar and vitamins

Qualitative analysis of fruit powder was carried out by using the standard procedure to determine the Sugars.[31]

RESULTS

The presence of Inorganic elements and specified physicochemical parameters were determined (Table-1). The TLC Profile of successive solvent extracts is a fundamental module of the contemporary monograph was depicted in table 2. The taste of the fresh fruit was sugary. The Qualitative chemical test report was shown the presence of Glucose, Fructose were the sources of carbohydrate present in the fruit. The secondary metabolites such as tannin, phenolic compounds and flavonoids, and sterols are the secondary metabolites in the fruit. The behaviors of powder with different chemical reagents are depicted in table 3. In fluorescence analysis, Fruit pulp powder was treated with different alkalis and acids had undergone the short and long UV examinations. The results show 1NHCl given pale yellow and brownish yellow and 1N NaOH produced yellow, brown and yellowish green and 50% HNO_3 turned green and greenish brown, 1N NAOH in Methanol produced dark brown and brownish green and 50% H_2SO_4 given pale reddish and reddish brown and Methanol in nitrocellulose turned yellow and dark green under short and long UV respectively.

Table1. Physicochemical values of fruit of *Elaeocarpus oblongus*

Parameters	Results
1. Organoleptic characteristics.	
Appearance	Coarse powder
Colour	Greenish white
Odour	Characteristic
2. Loss on drying	12%w/v
3. pH values	
pH of 1% aqueous solution	5.2
4. Ash values (%)	
Total ash	2.66%
Acid insoluble ash	0.66%
Sulphated ash	3.033%
Water soluble matter (%)	31.068%
Alcohol soluble matter (%)	30.94%
5. Successive solvent extractives (%)	
Hexane extract	0.23%
Chloroform extract	0.25%
Ethyl acetate extract	0.47%
Ethanol extract	15.66%
Aqeous extract	10.40%
6. Foreign organic matter	0.5% w/w
7. Elemental analysis	
In organic Elements	Quantity of elements (µg/g) in dried powder
Zn	46.2
Mn	53.3
Cu	7.5
Cr	4.9
Pb	14.3

Studies on Microscopic characters of *Elaeocarpus oblongus*

The fruit is a drupe with stony tuberculate pericarp. The seeds have a hard seed coat. The pericarp has a thin epidermal layer of small cells which are thick walled. The mesocarp very wide, comprises entire cells being thick walled, homogeneous and the cells have tannin contents (Figure 1). When the fruit matures the cells of mesocarp become thick walled and lignified sclerides scattered in the mesocarp are small clusters of vascular bundles which comprise a few xylem and phloem elements (Figure 2.1).

In the young fruit small clusters of lerachyscelerides are seen sparingly distributed in the mesocarp (Figure 2.2). The ground parenchyma cells of the mesocarp are polygonal in the outer zone (Figure 1) and the cells become gradually oblong in the inner zone.

Endosperm

Figure 3.3 depicted the endosperm tissues are thick towards the micropylar end and gradually become thinner towards the chalaza end (Figure 2.3). The endosperm is 1.7 mm thicker at the micropylar end and less than 1mm through the opposite part. The endosperm has fairly thick epidermis comprising very vertically oblong cells. The cells inner in the epidermis are squarish, thin walled and compact towards the inner part of the endosperm, the cells get arranged in regular, compact parallel lines. All the cells possess some darkly stained spherical bodies of varying sizes; the chemical nature of these bodies not known.

Embryo

The embryo consists of flat, uniformly thick cotyledons. It consists of inner and outer, fairly distinct epidermal layers and about 8 layers of squarish compact parenchyma cells of ground tissue. The cells contain large, darkly stained bodies. The cotyledon is 170µm thick.

Table 2. TLC finger printing of different extracts of fruit of *Elaeocarpus oblongus*

Extract	Solvent system	Detection	Spots	hR_f Values
Hexane	Toluene: methanol (25:75)	Ceric sulphate- Sulphuric acid (65%)	5	04;34;52;58; 65
Chloroform	n-hexanae Acetone (20:80)	Ceric sulphate- Sulphuric acid (65%)	3	06; 16;20
Ethyl acetate	Toluene : Methanol (80:60)	Ceric sulphate- Sulphuric acid (65%)	3	12;22; 39
Ethanol	CH_2CL_2 Methanol (20:80)	Ceric sulphate- Sulphuric acid (65%)	4	06; 36;41;54

Table 3. Behavior of the powder with different chemical reagents of fruit of *Elaeocarpus oblongus*

Reagents	Observation	Chemical nature
Aqeous $Fecl_3$	Green colour	Tannins/Flavanoids Present
Dilute ammonia solution	No change	Anthraquinone absent
5%Aqeous KOH	No change	Anthraquinone absent
Aqeous $Hgcl_2$	No precipitate	Alkaloids absent
Picric acid	No precipitate	Alkaloids absent
Aqeous $AgNo_3$	No white precipitate	Protein absent
Conc H_2So_4	Reddish brown	Steroids/Triterpenoids present
Mg-Hcl	Magenta Yellow	Flavanoids present
Picric acid	No change	Alkaloids absent
Iodine solution	No change	Starch absent
Mayer's reagent	No precipitate	Alkaloids absent
Aqueous Lead aceate	white precipitate	Tannins present
Aqueous NAOH	Yellow colour	Flavanoids present

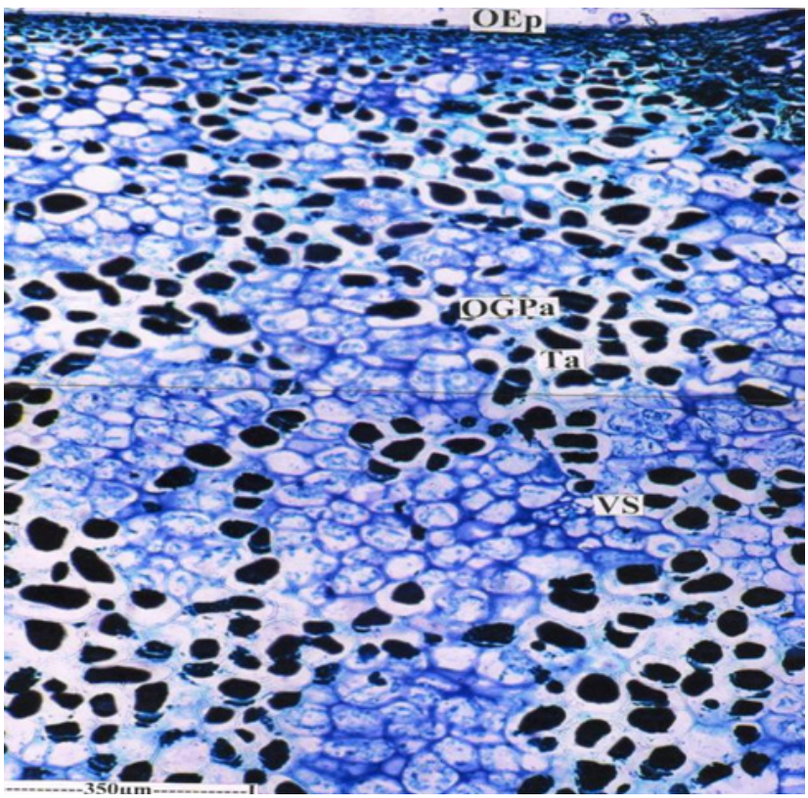

Figure 1. T.S. of Pericarp of the young fruit showing vascular strands and abundance of tanniferous cells. OEP – Outer epidermis, OGPA – Outer ground parenchyma, Ta- Tannin, VS- Vascular strand.

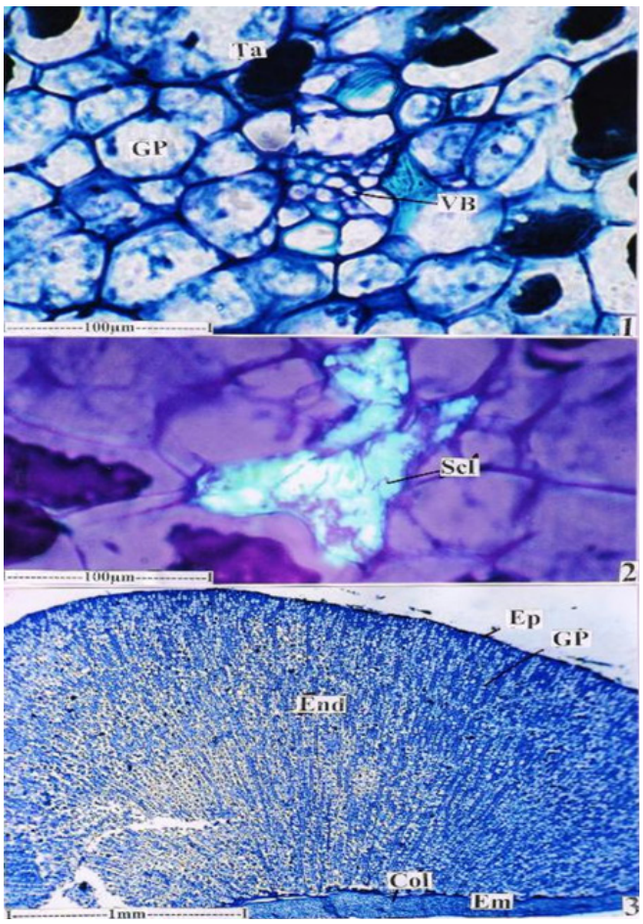

Fig. 2.1 A vascular strand of the pericarp enlarged.
2.2 A small cluster of sclereids in the pericarp (polarized microscopy).
2.3 T.S. of cotyledon.
End – Endosperm, Em – Embryo, EP – Epidermis, GP – Ground parenchyma, Scl – sclereids, Ta – Tannin, VB – Vascular bundle

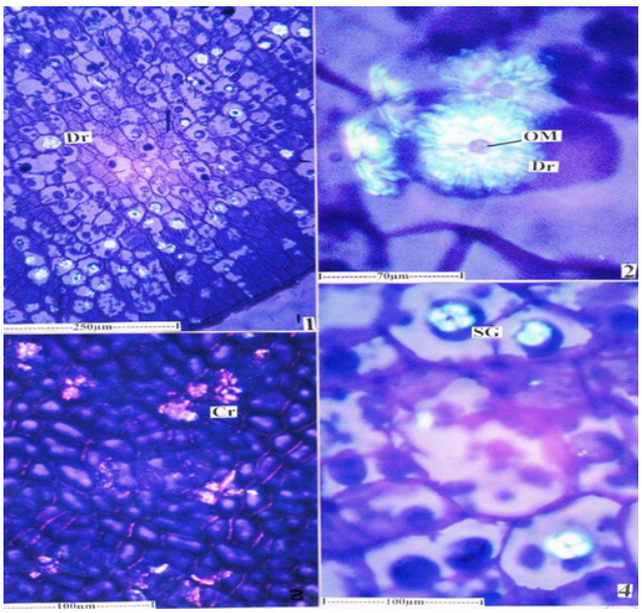

Fig 3.1 distribution of crystals in the endosperm cell.
3.2 A single rosette type of crystals.
3.3 Crystals in the pericarp of the fruits.
3.4 Starch grains in the endosperm.
Cr – crystal, Dr – druses, OM – Organic matter, SG – starch grain.

Crystal distribution

Calcium oxalate crystals fairly common in the endosperm cells (Figure 3.1, 3.2) and in the sclerotic mesocarp of the fruit (Figure 3.3). In the endosperm cells the crystals are rosette type, they are circular bodies with central dark organic matter (Figure 3.1, 3.2) some druses are also seen in the cells. The rosettes are 15 µm in diameter.

In the mesocarp of the fruit wall are seen small clusters of prismatic crystals (Figure 3.3). Starch grains are occasionally seen in the endosperm cells (Figure 3.4) they are circular and concentric.

Powder Microscopic characters

Microscopic study of powder revealed the presence of fragments of pericarp is seen in surface view. These fragments possess cells, which are polygonal in outline with very thick anticlinal walls. Some cell inclusions are also seen in the powder. Calcium oxalate crystals are located in the cells of the pericarp. Isolated parenchyma cells are abundant in the powder. The cells are irregular shape or spherical to elongated. They are thin walled and do not possess any inclusions.

Sclereids

Sclereids of different shape and size are abundant in the powder they are all lerachysclereids types. The sclereids are isodiametric, elongated, or irregular in shape; they have thick, lignified walls and narrow lumen. The walls have wide canal line pits, the sclereids are seen either isolated or in large masses.

Trichomes are non glandular, occasionally seen in the powder. They are unicellular, unbranched and thick walled. The lumen is wide. The trichome is gradually tapering towards the top, it is 700 µm long and 400 µm thick at the base. These quantitative data are useful for setting standards for crude drug.

Seed; Ovary are 2–5 celled ovules 2 in each cell. Styles subulate are entire. Drupe with a single bony tuberculate stone divided into 1–5 seeded cells. Seeds are pendulous, testa hard albumen fleshy cotyledons broad.[32]

DISCUSSION

In the view of developing a customary monograph for this species, the scanning electron microscope [SEM] studies of leaves of *Elaeocarpus oblongus* had been evaluated. The results show variation in the cuticular ornamentation compared to stomata and trichomes of *E.oblongus* with other species. These distinguished characters found to be useful in identifying the members of this species. The cuticular features were aiding in deciding the taxonomic positions of the number of Elaeocarpaceae family.[33]

A chemical character of the *Elaeocarpus* species almost habitually contains either myricetin or gallic acid. Four out of seven species have been found to contain mearnesetin, rare methyl ether of myricetin. The leaf of the *Elaeocarpus oblongus* contains mearnsetin (flavonoid) 00.015% and myricetin, 00.19% flavonoid.[2] The qualitative analysis report showed (Table 3) the presence of primary metabolites such as Carbohydrate in the form of glucose and fructose in the fruit. Phenolic compounds, tannin, flavonoids, saponins and sterols and fatty acids were the secondary metabolites in the fruit.

Leaf, stem and stem bark of *Elaeocarpus oblongus* extracts was evaluated biologically for various activities such as antibacterial, antiviral, antifungal and effect on respiration and effect on cardiovascular and effect on isolated ileum and effect on isolated rat uterus and effect on CNS and cross behavior and effect of hypothermia analgesia and supra maximal electroshock seizure pattern test and diuretic and anti inflammatory. It was reported that the LD_{50} of the leaf, stem and stem bark was 464 mg/kg, 1000 mg/kg and 175 mg/kg respectively.[34] Stem and stem bark was not showing any potential biological activity, only leaf exhibited action on respiration. The current study has selected the much used edible fruit of this species for setting the quality control parameters. The fruits of *E. oblongus* exhibited potent antioxidant activity towards 1, 1-diphenyl-2- picrylhydrazyl free radical (DPPH·) (EC50 = 0.200 ± 0.76 and 0.250 ± 1.76 mg mL^{-1}), superoxide anion ($O2^{\cdot-}$) (EC50 = 0.50 ± 0.82 and 0. 50 ± 1.20 mg mL^{-1}), hydroxylion (˙OH) (EC50 = 0.250 ± 0.22 and 0.500 ± 0.48 mg mL^{-1}) and nitric oxide (NO) (EC50 = 0.250 ± 0.22 and 0. 250 ± 0.552 mg mL^{-1}).[35]

This fruit is susceptible to fungal growth since the fruit contains much moisture content was estimated about 12% w/w. In our antifungal studies, we had got a negative result, 10mg of the alcoholic extract of fruit in DMSO was failed to prevent the growth of *Candida albicans* in potato dextrose agar medium. However Mycorrhizal type and Dark septate endophyte (DSE) fungal association was not found in the root of *Elaeocarpus oblongus*.[36] Microscopic study of powder revealed the presence of rosette type of calcium oxalate crystals, lerachysclereids and squarish compact parenchyma cells of ground tissue are important anatomical characters and this observation were found to be identical features.

CONCLUSION

The physicochemical characters and anatomical characters, powder microscopy, and its quantitative data have been provided in this work can be used to develop the fundamental module of the contemporary monograph and also helped to identify and differentiate the closely allied species of the genus *Elaeocarpus*. The present study continuously progress to analyse the nutritive value and to isolate the important flavanoids in the fruit and screening the antinociceptive activity to prove folkloric report.

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CONFLICTS OF INTEREST

All authors, none to declare

ABBREVIATIONS

OEP – Outer epidermis, OGPA – Outer ground parenchyma, Ta – Tannin, VS – Vascular strand, IGPA – inner ground parenchyma, In – Integument, END – Endosperm, EM – Embryo, EP – Epidermis, GP – Ground parenchyma, SCL – sclereids, CT – cotyledon, DB – Dark stained bodies, IEP – Inner epidermis, DR – druses, OM – Organic matter, SG – starch grain, AW – Anticlinal wall, CR – crystal, EC – Epidermal cell, PA – parenchyma, NTR – Non glandular trichome, IUCN – International Union for Conservation of Nature, WHO – World Health Organization, QCMMP – Quality control methods for medicinal plant materials, PARC – Plant anatomy research centre, Zn – Zinc, Mn – Magnesium, Cu – Copper, Cr – Chromium, Pd – Lead, UV – Ultraviolet, RLS – Radial longitudinal section, TLS – Transverse longitudinal section, FAA – Formalin Acetic acid Alcohol, TBA – tertiary–Butyl alcohol, UV – Ultraviolet, EC50 – Effective

concentration, LD_{50} – Lethal Dose, CNS – Central nervous system, TLC – Thin layer chromatography, FAA – Formalin Acetic acid Alcohol, DMSO – Dimethyl sulphoxide, DSE – Dark septate endophyte, (DPPH·) – 1, 1-diphenyl-2- picrylhydrazyl free radical, ($O2^{\cdot-}$) – Superoxide anion, (·OH) – Hydroxyl ion, (NO) – Nitric oxide, nm – nanometer, mm – milimeter.

REFERENCES

1. Coode MJE. "Elaeocarpus in New Guinea - new taxa in the Debruynii subgroup of the Monocera group, Contributions to the Flora of Mt Jaya, V". Kew Bulletin, Kew: United Kingdom; 2001.
2. Lalchand S, Dasgupta S, Chattopadhyay SK, Ray AB. Chemical investigation of some Elaeocarpus species. Planta medica.1977; 32:197–9.
3. Gagan Shah, Prabh Simran Singh, Mann AS, Shri R. Scientific basis for the chemical constituent and therapeutic use of Elaeocarpus species: A Review. Int J of Insti Pharm and Life Sci. 2011; Jul-Aug 11:267–78.
4. Amid Dadhich, Anirudha Rishi, Gargi Sharma, Subhash Chandra. Phytochemicals of Elaeocarpus with their therapeutic value A Review. Int J Pharm Bio Sci. 2013 Jul; 43:591–8.
5. Nair NC, Henry AN. Flora of Tamilnadu. Howrah, India :Botanical survey of India; 1983.
6. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian Medicinal Plants, 3rd ed, New Delhi: Council of Scientific and Industrial Research; 1992.
7. Council of Scientific and Industrial Research (CSIR), The wealth of India: a dictionary of Indian raw materials and industrial products, Raw materials, revised edition, Publication and information directorate, New Delhi, India; 1992.
8. Pullaiah T. Encyclopedia of world medicinal plants. New Delhi: Regency Publication; 2006.
9. Jain SK. Dictionary of Indian folk medicine and ethnobotany. New Delhi: Deep publication; 1991.
10. http://municipality.tn.gov.in/ooty/abt-muni.htm
11. Statistical hand book-The Nilgiris. nilgiris.nic.in/images/districthandbook0809.pdf
12. PK Manigandan PK, Activity concentration of radionuclides in plants in the environment of Western Ghats. Iran. J. Radiat. Res., 2009; 72:85–90.
13. Sass JE. Elements of botanical micro technique. New York: Mc Graw Hill; 1940.
14. Johansen DA. Plant Micro technique. New York, USA: Mc Graw-Hill; 1940.
15. John ES. Elements of botanical micro technique. 1st ed. Mc Graw hill; NewYork: 1940.
16. Kraus JE, Arduim M. Manual basico de metodos em morfologia vegetal. Editora da universidade rural, Seropedica; 1997.
17. O'Brien TP, Feder N, Mc Cull ME. Polychromatic Staining of Plant Cell walls by toluidine blue. O.Protoplasma. 1964; 59:364–73.
18. Brain KR, Turner TD. The Practical evaluation of Phytopharmaceuticals. Bristol: Wright Scientechnica; 1975.
19. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi, India; Vallabh Prakashan; 2005.
20. Kay LA. The Microscopical Studies of Drugs. Ist ed. London: Bailliere & Cox; 1938.
21. Trease GE, Trease NC. Pharmacognosy. London: Crowell-Collier & Macmillan Publishers; 1972.
22. Wallis TE. Text Book of Pharmacognosy. 5th ed. London: J & A Churchill; 1967.
23. Easu K. Plant Anatomy John Wiley & sons. New York: John Wiley & sons; 1964 and Anatomy of seed Plants. New York: John Wiley & sons; 1979
24. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharm Assoc. 1949; 38:324–31.
25. Kokoski CJ, Kokoski RJ, Salma FJ. Fluorescence of powdered vegetable drugs under ultraviolet radiation. J Am Pharm Assoc.1958; 47:715–7.
26. Vogel AIC. A Text Book of Macro and Semi-Micro Qualitative inorganic analysis. London: Longman, Green- Co. Ltd; 1953.
27. World health organization. Quality Control Methods for Medicinal plants. Geneva; 1998.
28. Ayurvedic Pharmacopeia of India. Ministry of Health and Family Welfare, Department of Ayush, New Delhi; 1999.
29. Wagner H, Bladt S. Plant Drug Analysis In: A Thin Layer Chromatography Atlas. 2nd ed. Springer-Verlag: New York; 1996.
30. Stahl E. Thin layer chromatography. A Laboratory Handbook. 2nd ed. New York: Springer-Verlag Berlin; 1969.
31. Deb AC. Fundamentals of Biochemistry.7th ed. Kolkata: New Central Book Agency; 1998.
32. Gamble JS, Fischer CEC. Flora of Presidency of Madras. London: Adlard and Sons Ltd; 1924.
33. Singh HP, Dube VP. Scanning Electron Microscope studies of the leaves in the family Elaeocarpaceae. Phytomorphology. 1991; 41: 257–65.
34. Abraham Z, Bhakuni DS, Garg HS, Geel AK, Mehrotra BN, Patnaik GK, et al. Screening of Indian plants for biological activity. Ind J of Exp Bio.1986; 24:48–68.
35. Ramakrishnan S, Jagathala M, Engoor SA, Paul Hephzibah C, Mangalanandan SL et al, Antioxidant Capacity of *Rubus niveus* Thunb. and *Elaeocarpus oblongus* Gartn. Fruits: 2011. http://www.globalsciencebooks.info/JournalsSup/images/WebJournals/FOOD_5(SI1)IssueInformation.pdf.
36. Govindaraj, Bagyalakshmi, Thangavelu Muthukumar, Kullaiyan Sathiyadash, Vellaisamy Muniappan. Mycorrhizal and dark septate fungal associations in shoal species of Western Ghats, southern India. *Mycoscience*. 2010; Jan 511:44–52.