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Pharmacognostical analysis of *Toddalia asiatica* (L.) lam. (Rutaceae) fruit

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Abstract
*Toddalia asiatica*is a reputed ever green tree belonging to the family Rutaceae; commonly known as Indian tulip tree. The plant is distributed tropical regions and coastal forest in India. It is well known and all the parts are used in Indian system of medicine. The plant has been used as malaria, toothaches and bronchial pains. The scientific parameter is necessary to identify the exact plant material and to find its quality and purity. The present study deals with various pharmacognostical examinations like organoleptic or macroscopical characters, microscopical or anatomical studies were carried out and the parameters were reported. These studies indicated the possible information for correct identification and standardization of this plant material.

Keywords: *Toddalia asiatica*, fruit, microscopy and pahrmacognosticalevaluation

Introduction
Toddalia asiatica (L.) Lam. Is known as 'Milagarani' in Siddha system of medicine it is knowm as Kanchana in Ayurveda. *Toddalia asiatica* a very variable rambling, prickely, sarmentose shrub, distributed almost throughout India, ascending to an altitude of 2,500m. In south India, the plants are very common in the Nilgiri and palani hills and also in the scrubby jungles of Orissa. In the plains, particularly in dry situations, the plant assumes the form of a low shrub with smaller and narrower leaflets. Plant contains coumarins toddaalone, toddanolnorbraylin, and 5, 7, 8-trimethoxy coumarin. *Toddalia* is a monotypic genus of family Rutaceae [1, 2]. It is a flowering plant in the citrus family containing the single species *Toddaliaasiatica* commonly known as orange climber[2]. The botanical synonyms of *Toddaliaasiatica* (L.) Lam. includes *Paullinia asiatica*, *Toddalia aculeate*, *Toddaliaeffusa* and *Toddaliaambigua*. It is a liana with woody, corky, thorny stems that climb on trees, rea-ching up to 10 meters in length. It has yellow-green flowers, shiny green citrus-scented leaves, and orange fruits about half a centimeter in diameter that tastes like orange peel. The seeds are dispersed by birds and monkeys that eat the fruits within the forests [3].
Toddalia asiatica, is native to many countries in Africa and Asia and it grows in forested riparian habitat with high rainfall [4]. It is used as a folklore remedy for various ailments in many areas of the world. *T. asiatica*is used in treatment of several diseases that includes malaria [5, 2] stomach ache, chest pains, sore throat, food poisoning [2], cough, indigestion, nasal and bronchial pains [6]. Extracts of the plant have demonstrated antiviral activity against H1N1 influenza in the laboratory [7]. The harvest of this slow-growing plant from the wild for medicinal use may cause its populations to decline and protocols for domestication or propagation of the tree are being researched [4].
In Africa the plant is known by various local names: Ajua, Nyalwet (Luo), Katemwe (Tugen), Mkuro, Chikombe za Chui (Digo), Mdakakomba (Swahili), Mwikunya (Kikuyu), Olebarm-onyo (Maasai), Gwambadzi (Vhavenda SA), Ranklemoentjie (Afrikaan). The mode of propa-gation is by seed or root suckers [4]. Remedy for various ailments in many areas of the world. *T. asiatica*is used in treatment ofseveral diseases that includes malaria2, 5, stomach ache, chest pains, sore throat, food poisoning [2], cough, indigestion, nasal and Extracts of the plant have demonstrated antiviral activity against H1N1 influenza in the laboratory [7]. The harvest of this slow-growing plant from the wild for medicinal use may cause its populations to decline and protocols for domestication or propagation of the tree are being researched [4].
Medicinal plants are well-known natural sources for the treatment of various diseases since antiquity. About 20,000 plant species used for medicinal purposes are reported by World Health Organisation [8]. Despite emphasis being put in research of synthetic drugs, a certain interest in medicinal plants has been reborn, in part due to the fact that a lot of synthetic drugs are potentially toxic and are not free of side effects on the host. This has urged microbiologists all over the world for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents [9].
Toddalia asiatica Lam. (Family - Rutaceae) is a straggler, known as "Milakarani" in Tamil, Kanchana‟ in Sanskrit, and,, Kanj‟ in Hindi, used in Indian systems of medicine. There are two varieties; *Toddalia asiatica* var. gracilis and var. floribunda and they are found in the west and southern parts of India. Plants of var.*gracilis* are generally found in denuded slopes, waste lands and lower hills, whereas that of var. floribunda are found only on high altitudes. This plant grows well in clay soils and is widespread in Africa, Asia and Madagascar [10]. Milakarani has gained popularity amongst traditional health practitioners for treating numerous ailments.

Amongst the documented ethnomedicinal uses, the fruit of this plant is known to have been popularly applied intreating malaria, particularly in East Africa [2]. The plant has been used in the curing of diseases like paralysis, malarial and intermittent fever, dyspepsia, colic, diarrhea, cough, bronchitis, nausea, wounds, contaminated ulcers, epilepsy, gonorrhoea and general debility [12, 13]. These therapeutic values are due to the presence of coumarins, quinoline and benzophenanthridine alkaloids [14]. Several compounds were isolated from various parts and different extracts of *T. asiatica*. In 1965, Pakrashi and Bhattacharyya reported presence of alkaloids, dihydrochelerythrine and berberin, [15] reported toddalinine, robustine, skimmianine, dictamnine, bergapten, luvangetin and isopimpinelline. Toddasin, Toddanol and Toddanone have been isolated from *T. asiatica* [16, 17, 18] have documented coumarins, fatty acid esters, β-sitosterol, alkaloids and some other compounds. A new coumarin, Toddalenone [19] and an antiplasmodial coumarin were isolated from *T. asiatica* [20].
Fruits are eaten for relief from burning sensation in stomach [21]. Leaf and root are used to cure rheumatic swellings and fever pain. The root bark is used medicinally as a tonic and for the treatment of stomach ailments [10], while the leaves are used in patients having lung diseases or rheumatism and for the treatment of fever, asthma and respiratory diseases [10, 22]. The alkaloids of the crude extract have been shown to have anti-inflammatory effects in rats using the carragennan test [22] and to inhibit the auricle swelling caused by xylol and joint swelling caused by agar in rats [24]. *T. asiatica*are reported to have anti-malarial and anti-leukimatic properties [25, 26]. The central and peripheral antinociceptive effects of *T.asiatica* have been demonstrated using mice [27].

Materials and Methods

Plant collection and authentication

The plant specimens *(Toddalia asiatica var. floribunda*) fruit were collected from Indira Nagar, Emerald, Udhagamandalam, The Niligiris, Tamil Nadu, India. Care was taken to select healthy plants and normal organs.The plant material was taxonomically identified and authenticated by Director, Plant Anatomy Research Centre, Chennai A voucher specimen (PARC/250/07) has been depositedin the herbarium Department of Botany, Periyar E.V.R. College, Tiruchirappalli - 620 023,, Tamil Nadu, India.

Pharmacognostical studies

Organoleptic or Macroscopical character

The fresh young (green in colour) and mature fruit (Yellow in colour) were also subjected to investigation. Studies, such as shape, size, outer surface, inner surface, fracture, taste and odour of fruit, were carried out.

Microscopical studies

Preparation of specimen

The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml).After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule [13]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60º C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning: The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure[28]. The sections were stained with Toluidine blue as per the method [15]. Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green and IKI(for Starch). For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid [28] were prepared.
Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with Naoh and mounted in glycerine medium after staining. Different cell component were studied and measured [29].

Photomicrographs: Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books [30, 31].

Results and Discussion

Identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive. Standardization plays an important role in the production of phyto pharmaceuticals of standard quality and purity

Microscopic investigation: It is a liana with woody, corky, thorny stems that climb on trees, rea-ching up to 10 meters in length. It has yellow-green flowers, shiny green citrus-scented leaves, and orange fruits about half a centimeter in diameter that tastes like orange peel.
.In the present study the fruit of *Toddalia asiatica* var. *floribunda* (fruit) was evaluated for pharmacognostic analysis which revealed the following results.

Anatomy of the fruit: is subglobose, 3-6 celled, fleshy; Seeds are one in each cells (Fig: 1, 2). The fruit is a berry with thick fleshy pericarp (Fig: 3, 4).

Locules: The locules are wide separated from each other by thick septa. The fruit consists of an epidermis (exocarp), an inner thick, parenchymatous mesocarp (Fig: 3).

Exocarp: The exocarp includes two or three layers of tangentially stretched thick walled cells. There are wide circular secretary cavities which originate by lysigenous process of mesocarp cells. The cells surrounding cavity are like epithelial cells. Some darkly stained spherical tannin bodies, are seen in and around the cavities (Fig: 5).

Fibres: Small and large clusters of fibres' and circular vascular bundles and tanninferous, Cells are seen in the pericarp (Figure: 6). Macroscopic characteristics Macroscopic characteristics of *Toddalia asiatica*var. *floribunda* (fruit) is shown in Table 1.

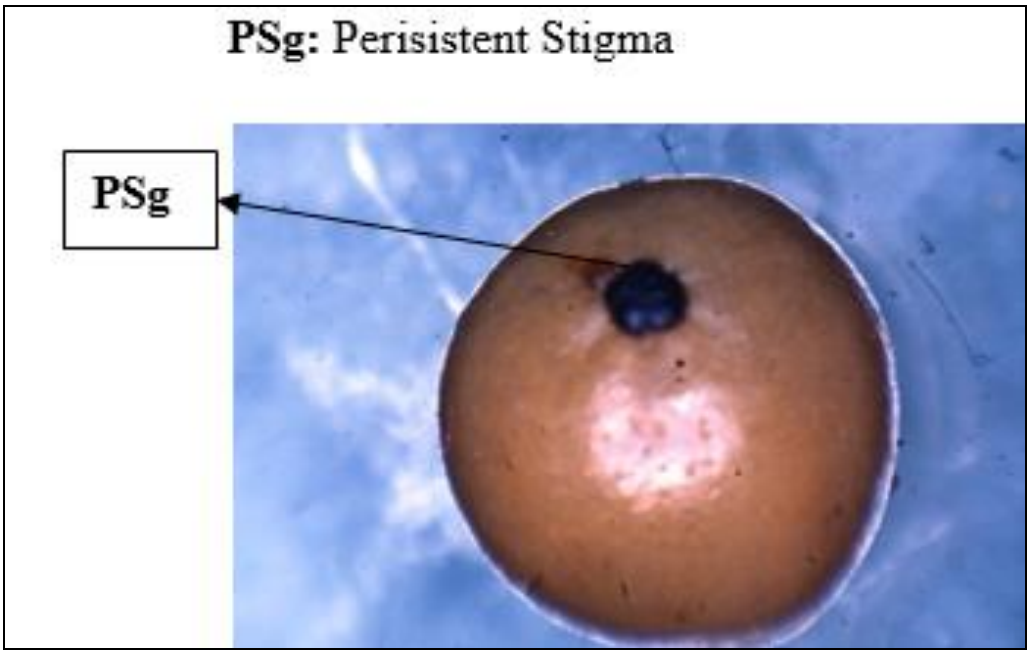

Fig 1: T. S of *Toddalia asiatica*var. *floribunda* (fruit)

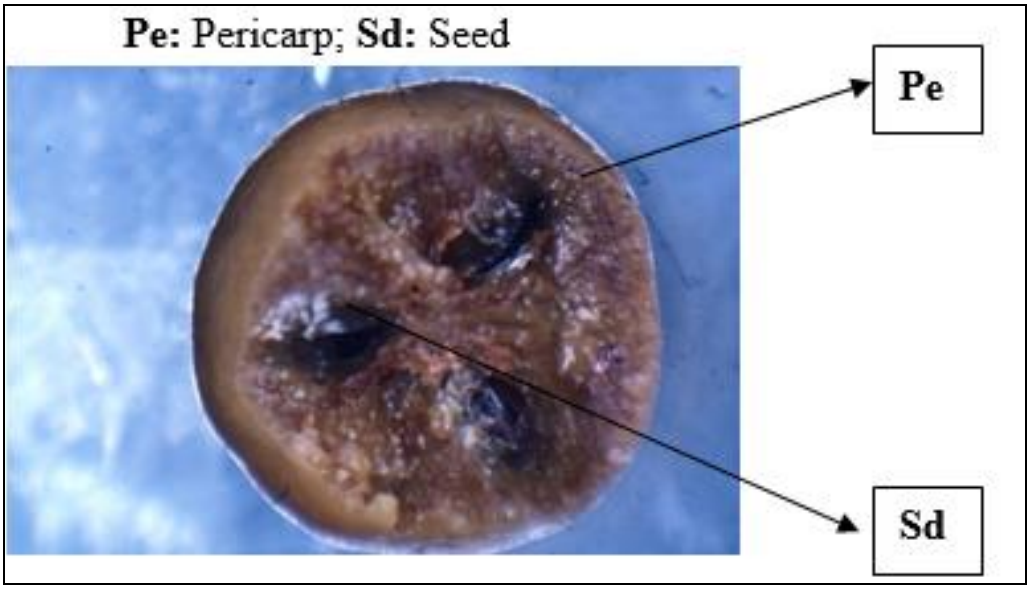

Fig 2: Fruit entire surface view and Cross Section view pf the fruit with seeds

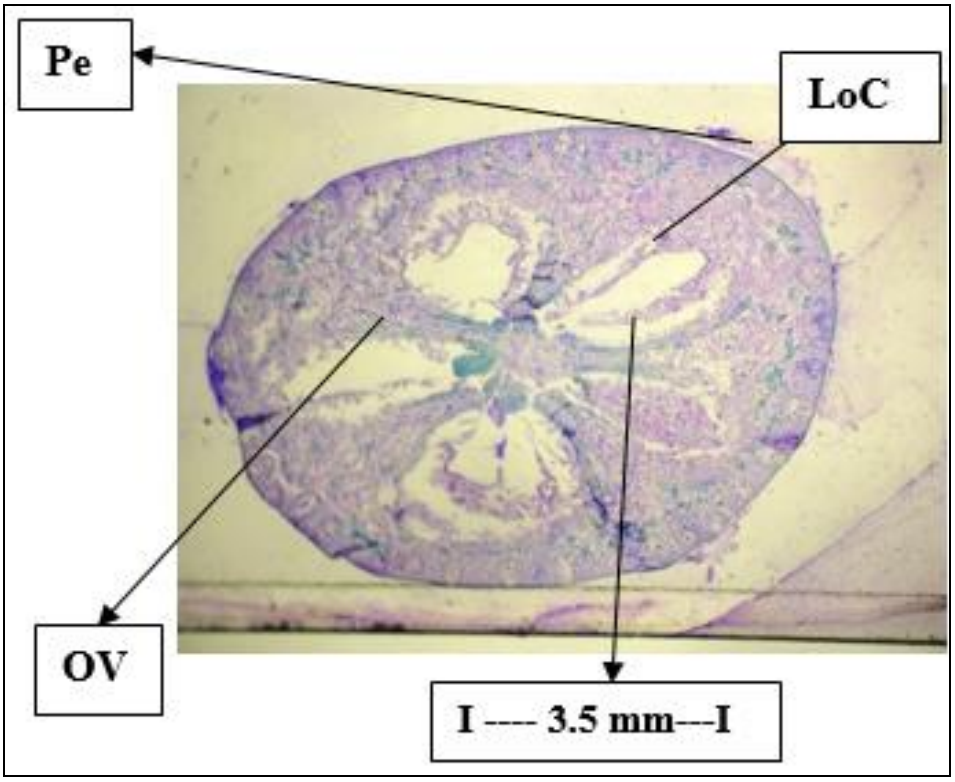

Fig 3: T.S. of fruit LoC: Locule chamber; Pe: Pericarp; LoC: Locule chamber; Pe: Pericarp;

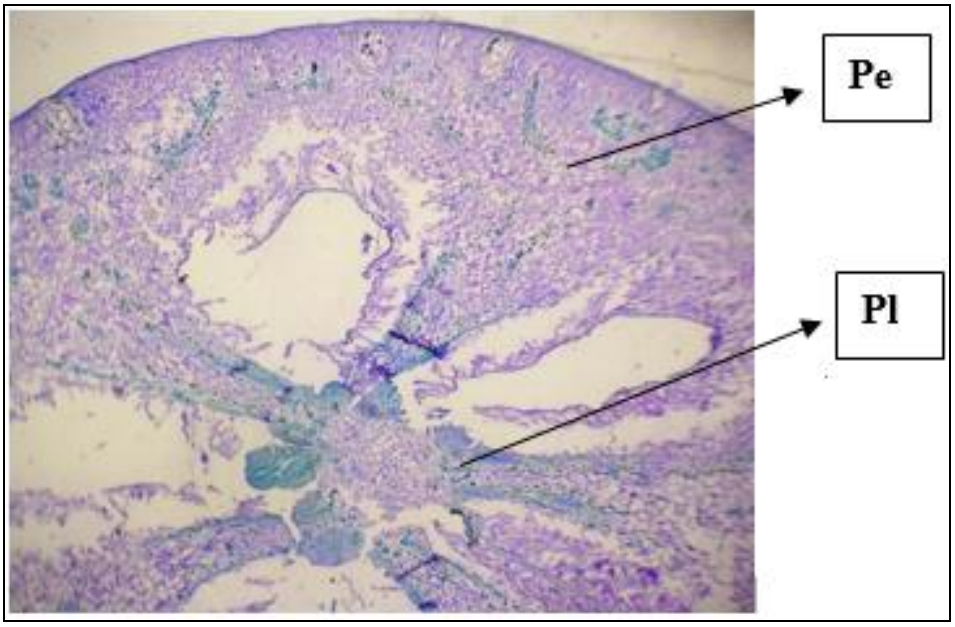

Fig 4: Secretary Cavity Se: Septal Partition; OV: Ovule OV: Ovule; Pl: Placentum; Se: Septal Partition

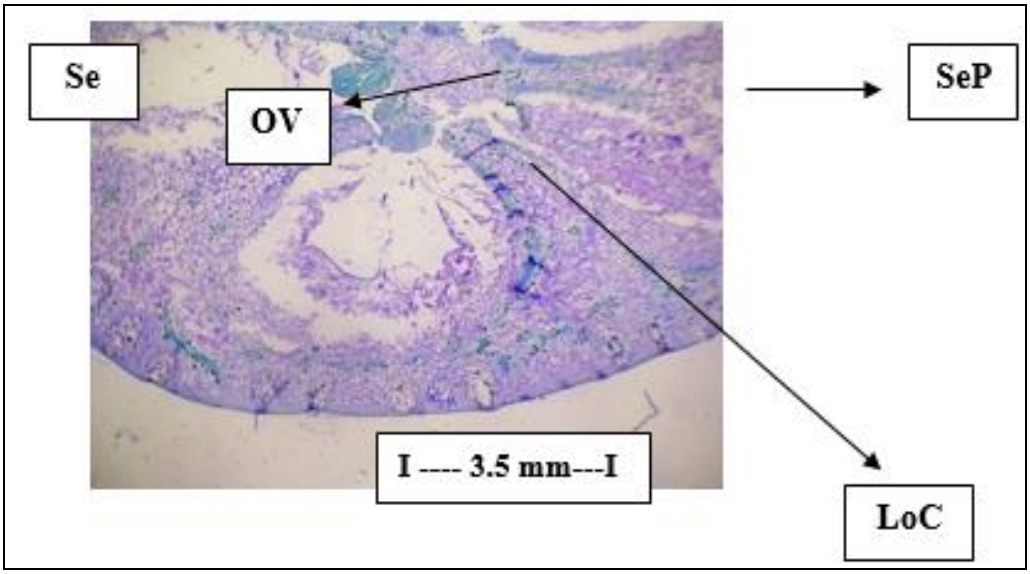

Fig 5: T.S of fruit - Pericarp portion

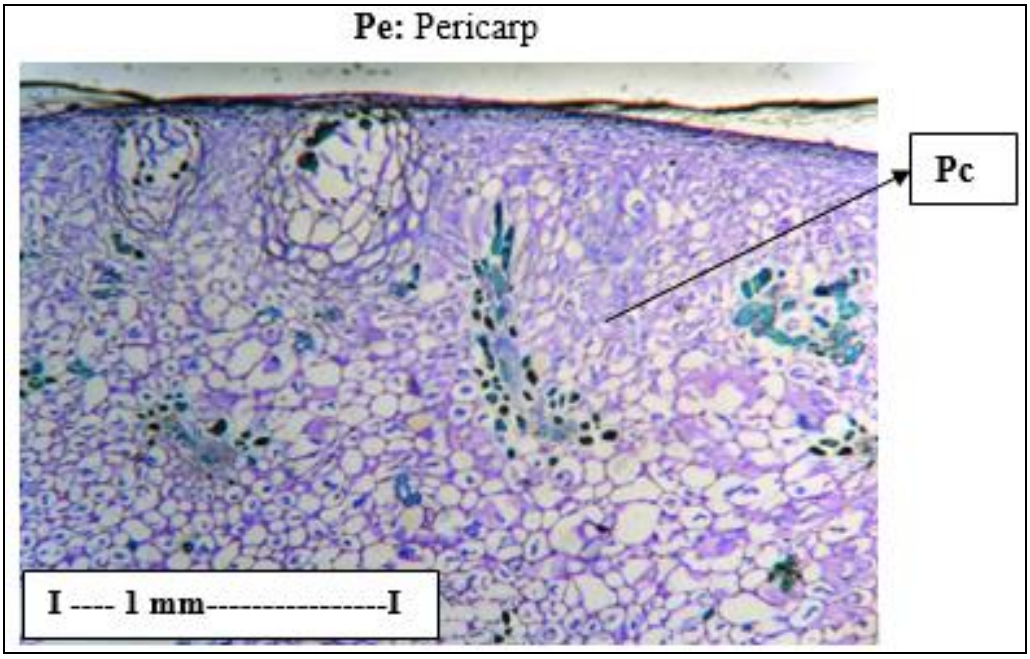

Fig 6: Secretary Cavity - Enlarged view Ep: Epidermis; SC: Secretary Cavity; EpC: Epithelial cells

Table1: Macroscopic characteristics of *Toddalia asiatica*var. *floribunda* (fruit).

S. No.	Parameters	Observation of fruit
1.	Color Young fruit - Green	
		Mature fruit - Yellow
2.	Odour	Characteristic
3.	Taste	Pungent
4.	Nature	Coarse powder

Conclusion

After the present investigation, it can be concluded that the Pharmacognostical studies of the leaves and stem from *Toddalia asiatica*var. *floribunda* (fruit) yielded a set of qualitative and quantitative arameters or standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant materials for future studies. These parameters also will serve as standard data for quality control studies of pharmaceutical preparations from the leaves *Toddalia asiatica*var. *floribunda* (fruit).

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References

1. Santapau H, Henry AN. A Dictionary of the flowering plant in India. Council of science and Industrial research New Delhi A Dictionary of the Flowering Plants in India. Reprint, First published in,1973-1994:198.
2. Orwa JA, Ondiko IJO, Minja RJA, Bekunda M. Use of *Toddalia asiatica* (L)Lam. (Rutacae) in traditional medicine practice in East Africa. Journal of Ethnopharmacology,2008:115:257-262.
3. Bussmann RW, Gilbreath GG, Solio J, Lutura M, Lutuluo R, Kunguru K *et al*. Plant use of the Maasai of Sekenani Valley, Maasai Mara, Kenya. Journal of Ethnobiology Ethno medicine,2006:2:22.
4. Nabwami J, Tabuti J, Bekundai MA. Characterization of the natural habitat of *Toddaliaasiat-ica* in the Lake Victoria basin: soil characteristics and seedling establishment. African Crop Science Conference Proceedings,2007:8:2057-2061.
5. Jeruto P, Mutai C, Ouma G, Lukhoba C. An inventory of medicinal plants that the people of Nandi use to treat malaria J. Anim. Plant Sci,2011:9:1192-1200.
6. Kokwaro. J.O. Medicinal Plants of East Africa. Nairobi, 2nd edition. East African Lit-erature Bureau, Nairobi, 1993, 222-223.
7. Lu SY, Qiao YJ, Xiao PG, Tan XH. Identification of ant-viral activity of *Toddalia asiatica*against influenza type A virus. ZongguoZong Yao Za Zhi,2005:13:998-1001.
8. World Health Organization (WHO). Traditional Medicine Strategy. WHO Publications, 2002-2005, 1-6.
9. Balasubramaniam LA, Manivannan R, Paramaguru R, Vijayakumar M. Evaluation of Anti-inflammatory and Antioxidant Activities of Stem Bark of *Todalia asiatica* (L) Lam. Using Different Experimental Models. Global Journal of Pharmacol,2011:5(2):67-72.
10. Watt JM, Breyer-Brandwijk MG. Medicinal and Poisonous Plants of Southern and Eastern Africa. E. & S. Livingstone Ltd.:Edimburgh & London, 1962.
11. Orwa JA, Jondiko IJ, Minja R J, Bekunda M. The use of *Toddalia asiatica* (L) Lam. (Rutaceae) in traditional medicine practice in East Africa. J Ethnopharmacol,2008:115:257-262. 36.
12. Kirtikar KR, Basu BD. Medicinal Plants of India. Pub. L.M. Basu, Allahabad, 1933, 3.
13. Chopra RN *et al*. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi, 1956, 174.
14. Bandara BMR, Hewage CM, Jayamanne DHIW, Adikaram NKB, Bandara KANP, Pinto MRM. Biological activity of some steamdistillates from leaves of ten species of Rutaceous plants. Journal of National Science Council of Sri Lanka,1990:18(1):71-77.
15. Deshmukh *et al*, Characterization of collagen and its precursors synthesized by rabbit-articular-cartilage cells in various culture systems.Eur J Biochem,1976:69(1):117-23.
16. Sharma HR, Ingalls JR, Delvin TJ. Apparent digestibility of Tower and Candle rapeseed meals by Holstein bull calves. Can. J. Anim. Sci,1980:60:915-918.
17. Sharma NP, Shoeb A, Kapil RS, Popli S *Toddalidimerine* P. a dimeric benzophenanthridine alkaloid from *Toddalia asiatica*. Phytochemistry,1981:20:2781-2783.
18. Reisch J, Strobel H. New constituents from *Toddalia aculeate* Pers., Rutaceae, Pharmazie,1982:37(12):862-83.
19. Ishii H, Kobayashi JI, Ishikawa T. Toddalenone: a new coumarin from Toddalia asiatica (*T. aculea*ta) structural establishment based on the chemical conversion of Limettin into Toddalenone. Chemical Pharmaceutical Bulletin,1983:31:3330-3333.
20. Oketch-Rabah HA, Mwangi JW, Lisgarten J, Mberu EK: A new antiplasmodial coumarin from Toddalia asiatica roots. Fitoterapia,2000:71:636 -640.
21. Ramachandran VS, Nair VC. Ethnobotanical observations on Irulars of Tamil Nadu (India) J. Econ. Tax Bot,1981:2:183-190.
22. Gurib-Fakim A, Gueho J, Bissoondoyal MD. PlantesMedicinales de Maurice. Editions de l'OceanIndien, Rose Hill, Mauritius. Tomes,1995-1997:1(2):3.
23. Balasubramaniam LA, Manivannan R, Paramaguru R, Vijayakumar M. Evaluation of Anti-inflammatory and Antioxidant Activities of Stem Bark of *Todalia asiatic*a (L) Lam. Using Different Experimental Models. Global Journal of Pharmacol,2011:5(2):67-72.
24. Hao XY, Peng L, Ye L, Huang NH, Shen YM. A study on anti-inflammatory and analgesic effects of alkaloids of *Toddalia asiatica*. Journal of Chinese integrative medicine,2004:2(6):450-452.
25. McCurdy, C. R., and Scully, S. S. Analgesic substances derived from natural products: Natureceuticals. Life Science,2005:78(5):476-484.
26. Schlage C, Mabula C, Mahunnah RLA, Heinrich M. Medicinal plants of the Washambaa (Tanzania): Documentation and Ethnopharmacological Evaluation. Plant Biol,2000:2:83-92.
27. Kariuki HN, Kanui TI, Yesesew A, Patel NB, Mbugua PM. Antinociceptive activity of*Toddalia asiatica* (L) Lam. in animal models of central and peripheral pain. Phytopharm-acology,2012:3:122-129.
28. Sass JE. Elements of Botanical Microtechnique. McGraw Hill Book Co; New York, 1940, 222.
29. Johansen DA. Plant Microtechnique. Mc Graw Hill Book Co; New York, 1940, 523.
30. O'Brien TP, Feder N, Mc Cull ME. Polychromatic Staining of Plant Cell walls by toluidine blue-O.Protoplasma,1964:59:364-373.
31. Easu K. Anatomy of seed Plants. John Wiley and sons. New York, 1979, 550.