International Journal of Pharmacy

Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

Pharmacognostic and Preliminary Phytochemical Screening of *Ocimum sanctum Linn.* Stem (Holy Basil), A Known Indian Folk Medicinal Plant

Aanchal Gwari*, Arvind Negi, Manish Mishra

Department of Pharmacognosy, GRD Institute of Management and Technology, Uttarakhand, India

***Corresponding author email: aanchalgwari@gmail.com**

Received on: 22-09-2021; Revised on: 06-10-2021; Accepted on: 13-10-2021

ABSTRACT

In recent years, attention has been turned to researchers to discover new alternative sources of anti-inflammatory agents, especially of plant origin. *Ocimum sanctum* (tulsi) has served humanity as a source of medicinal agents since its inception. Phytoconstituents present in leaves, bark, stems and flower spikes and to characterize the compounds responsible for the anti-inflammatory activity. Phytochemical screening of the stems of the plant reveals the presence of saponins, alkaloids, flavonoids, steroids, phenols, tannins and glycosides. *Ocimum sanctum* also known as tulsi or holybasil is an aromatic plant and belongs to the *Lamiaceae* family. It is widely used as a medicine to treat various ailments. The aim of the study was to analyze the various phytochemical components of the tulsi stem. The dried tulsi stem powder (100 g) was placed in the thimble of the soxhlet apparatus and the experiment was conducted separately for aqueous, methanol and ethanol. The percent yield was 6.0% w/w, 8.0% w/w and 9.0% w/w, respectively. The study reveals that various secondary metabolites such as carbohydrates, tannins, flavonoids, saponins, glycosides, terpenoids, fatty acids and phenol are present in the tulsi stem extract.

These phytochemicals are known to possess antiseptic, analgesic, anti-inflammatory, antimicrobial, anti-stress, immune-modulatory, hypoglycemic, hypotensive, and antioxidant properties. Therefore, it is more beneficial to use *tulsi asan* as a herbal medicine than as a chemically synthesized medicine.

India has a rich heritage of medicinal plants, Indian medicine systems use 80 percent of material derived from plants. Large quantities of plants are used in various systems of medicine practiced in India and in local health traditions for the treatment of human diseases since time immemorial. The use of tulsi as a source of medicine in humans has been in vogue since ancient times. Tulsi (*Ocimum sanctum Linn*.) It has been used for thousands of years in ayurveda for its various forms of healing property. Tulsi is legendary and the "incomparable" of India. It is one of the holiest and most appreciated of the many healing and healthy herbs of the East. Morphological and anatomical the characters play a fundamental role in the standardization of drugs. It is missing and adulterants are often passed off as real drugs. Currently research, *Ocimum sanctum* was selected for standardization due to its medicinal properties importance.

The present research aims to document the morphology, distribution, phytochemistry and medicinal properties of *Ocimum sanctum* and its future prospects for the further scientific investigation for the development of effective therapeutic compounds.

Keywords: *Ocimum sanctum*, Phytochemical, Medicine, Phytochemistry

INTRODUCTION

Currently, the World Health Organization (WHO) recognizes that herbal medicine is practiced throughout the world as an essential component of primary health care. According to the WHO, more than 80% of the world population still depends on medicinal herbs as the main source of medical treatment. In the system of traditional medicine, herbal medicines and formulations are used only for the purpose of ancient times. It is the morbid state of many herbs and plant products that do not have a significant anti-inflammatory action. However, the drug status is not based on the pharmacological evaluation of such herbal products for the claimed anti-inflammatory effects in traditional medicine [1].

Ocimum sanctum Linn. commonly known as holy basil (tulsi) is a perennial herbaceous plant, belongs to the *Lamiaecae* family and is considered one of the most important sources of medicines and drugs with many secondary metabolites and essential oils recommended for the treatment of malaria, diarrhea, bronchial asthma, dysentery, bronchitis, skin diseases, arthritis, eye pain, chronic fever and eye disease, etc. Furthermore, *Ocimum sanctum Linn.* has anticancer, antifungal, antimicrobial, antifertile, hepatoprotective, antispasmodic, cardioprotective, antiemetic, antidiabetic, analgesic, adaptogenic, anti-inflammatory and diaphoretic properties. The pharmacological studies reported in this survey confirm the therapeutic value of *O. sanctum*. Therefore, the present study analyzes the extraction and preliminary phytochemical analysis of *O. sanctum* Stem [2].

Plant profile

Plant-*O. sanctum* (Figures 1 and 2).

Figure 1: Krishna tulsi.

Figure 2: Ram tulsi.

Plant taxonomy

Varieties (Dravyaguna vol-II) Bhavaprakasha and Raja Nighantu have reference of two varieties (Tables 1-3).

Plant taxonomy		**Other names (Dravyaguna vol-II)**	
Kingdom	Plantae	English name	Holy basil/ sacred basil
Division	Magnoliophyta	Hindi name	Tulsi
Class	Magnoliophyta	Sanskrit name	Tulasi
Order	Lamiales	Gujarati name	Tulsi
Family	Lamiaceae	Telugu and Marathi	Tulasi
Genus	*Ocimum*	Malayalam	Tulasi
Species	*Sanctum*	Kennada name	Sri Tulasi
Binomial name	*Ocimum tenuiflorum* or *Ocimum sanctum L.*	Bengali	Kalotulsi, kural

Table 1: Plant taxonomy.

Plant	**Variety**
Shukla Tulsi	White variety-*Ocimum Americanum Linn.*
Krishna Tulsi	Black Variety-Purple leaf Basil (*Ocimum tenuiflorum)*
Vana Tulsi	Wild Green leaf tulsi (*Ocimum basilicum* and *Ocimum gratissimum*)
Holy basil (Rama Tulsi, Kapoor Tulsi)	Green leaf basil (*Ocimum sanctum Linn*, *Ocimum tenuiflorum*)

Table 2: Varieties of tulsi.

Phytochemical constituents

Tulsi is very complex, it contains many nutrients and other biologically active compounds. The nutritional and pharmacological properties of the whole herb naturally, as it has traditionally been used, are the result of synergistic interactions of many different active phytochemicals.

Ethanobotanical uses

Traditionally, various parts of plant are used as medicinal purpose.

It is part of alternative medicine and is found in many Ayurvedic preparations [3].

Tulsi leaves: relieve nasal congestion, nerve tonic, strengthen immunity, improve memory, revive mouth ulcers and infections, skin disorders such as ringworm, use dried leaf powder for dental health, care for eyes irritated and night blindness, stomach to promote appetite, expectorant, Diarrhea.

Tulsi tea or decoction: Prevents malaria, dengue, the common cold, acute fever.

Tulsi leaf juice: As a gargle to treat sore throat, Taken with honey for six months it can expel kidney stones, Lowers cholesterol, Cardioprotective, Beneficial in respiratory disorders (asthma, bronchitis, cold, flu, cholera).

Tulsi seeds: Good Antioxidants, Mucilaginous, Demulcents, Antiulcers, Antiemetics, Antidiarrheals [4].

Tulsi root: Malarial fever, Relieves pain from insects and stings, Prevents premature ejaculation.

Tulsi stem: Anticonvulsant, Antidiabetic, Anti-inflammatory, Antistress, cardioprotective, memory enhancer.

Therpeutic uses: Antidiabetic, cardiac activity, healing activity, radioprotective, genetic toxicity, antioxidant, lipid-lowering, antimicrobial, effect on gene transcription, gastroprotective, immunomodulatory effect, effect on the central nervous system, analgesic, antifertility, anthelmintic, anti-inflammatory activity , antitumor, thyroid activity.

Other uses: Urticaria, itching, rash, sinusitis, headache, use of the tulsi plant for severe respiratory problems, to smoke regularly, for ringworm, relieves vomiting, useful in infectious eye disorders, relieves the difficulty of urinating, improves digestive strength, improves taste, relieves anorexia, astringent, useful in repeated hiccups, etc [5].

MATERIAL AND METHODS

Collection and authentication of plant

Healthy, disease free, mature stems of *O. sanctum* were collected from the GMS Road region, Dehradun, Uttarakhand India, washed with sterile water and dried in the shade.. The collected plant material was identified and authenticated by scientist D/HOO Kumar Ambrish,

Table 3: Phytochemical constituents.

Plant parts	**Phyto-chemical constituents**
Leaf	The leaf volatile oil contain Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), Euginal , Urosolic acid, Carvacrol, Linalool, Limatrol, Caryophyllene, Methyl, Carvicol, (Estragol), Bornyl acete, Candinene, Camphene, Camphor, Carvacrol, B-Caryophellene, Humelene, Sesquiterpine hydrocorbone Caryophyllene. Two Flavonoids Orientien and Vicenin from aqueous leaf extract of ocimun sanctum have been isolated. (lllustrated Dravyaguna Vijnana, Vol. ll, by dr. JLN Shastry).
Seed	Seeds volatile oil have fatty acids and sitosterol; Linoleic acid, Linolenic acid, Oleic acid, Palmitric acid, Stearic acid, Dilinoleno-linolins, Linodilinolin, Hexoureic acid, in addition, the seeds mucilage contains some levels of sugars and anthocyans are present in green leaves. No caffeine or other stimulant are available.
Stem	Rosmarinic acid, Euginol, Propronic acid, Apigenin, Cirsimaritin, Isothymusin and Isothymonin. Two water-soluble Flavonoids: Orientin and Vicenin.Saponins, Flavonoids, Titerpenoids, and Tannins.
Whole Plant	Tannins, Alkaloids, Steroids, Phenols, Flavonoids, Resins and Vita-C, Vita-A, Vita-E, Zink. Calcium, Iron, Phosphours, Copper, Nickel, Carotene, Chrominum

Department of Botanical, India Botanical Survey, North Region Center, 192, Kaulagarh Road, Dehradun, India, saw reference no. 614. Where the voucher sample was preserved [6].

Preparation of leaf material

Stems of selected plant were plucked and washed thoroughly with running tap water. It was again washed with sterile distilled water to remove dirt prior to drying process. The Stem were dried in shade at room temperature for a week to remove the moisture content and powdered using mixer grinder. Finally, powdered sample was stored at room temperature for further studies [7].

Preparation of plant extract successive extraction using soxhlet apparatus: 100 g of powdered sample was taken in air tight bottles. To this, 100 ml of different solvents such as aqueous, ethanol, methanol, and distilled water was added. After weighed accurately 100 gm of dried drug in shaded (dried crushed flowers) and put into thimble made up of filter paper. Placed the thimble containing drug into soxhlet apparatus and solvents was added slowly onto it which was passed through the thimble and collected into round bottom flask. Here, 3 cycles were done and assembly was operated for 6 hours and temperature not exceeding not more than 60°C. Successive extraction was done by using solvents in the order-aqueous, methanol and ethanol. After completed the extraction process, the heating was stopped and the mixture of the liquid was collected and placed into china dish. Evaporated the mixture up to dryness into water bath. Cooled properly and stored in a well closed container and kept at 4°C. The extract obtained after successive extraction are further used for performing preliminary phytochemical screening, TLC fingerprinting analysis of the stem of *Ocimum sanctum*. The percentage yield of the extract was calculated using the following formula:

Percentage yield=final weight of the dried extract/Initial weight of the powder × 100

All the three extracts were kept in separate vials in the refrigerator till further use [8].

Macroscopic characters of *Ocimum sanctum (Linn.)* stem

(Figure 3 and Table 4).

Figure 3: *Ocimum sanctum (Linn.)* stem.

S.No	Characters	Observations
1	Colour	Externally purplish brown to black, Internally cream coloured,
2	Odour	Faintly aromatic
3	Taste	Characterstic
4	Occurence	Erect, Hairy and woody bear simple toothed
5	Xylem	Fracture, Fibrous in bark and short in xylem
6	Diameter	10-15 cm

Table 4: Macroscopic characters of *Ocimum sanctum (Linn.)* stem.

Microscopy of Ocimum sanctum (Linn.) stem

Powder microscopy: (Figures 4-11).

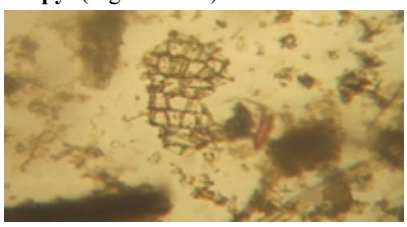

Figure 4: Cork cells.

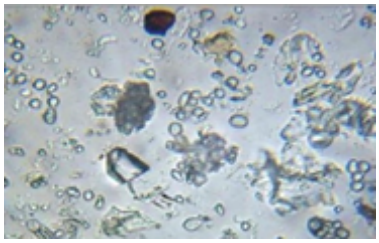

Figure 5: Starch grains and prismatic crystals.

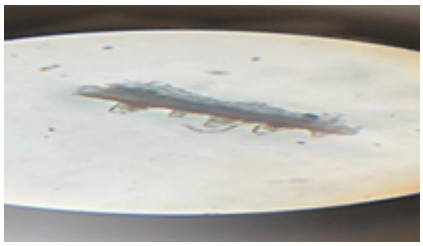

Figure 6: Fiber.

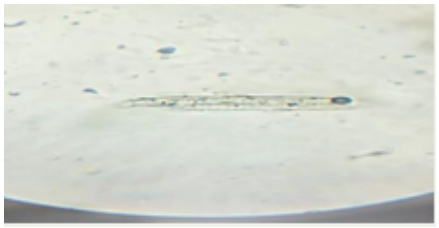

Figure 7: Xylem fiber.

Figure 8: Bordered pitted vessels.

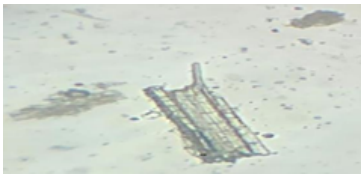

Figure 9: Xylem vessel.

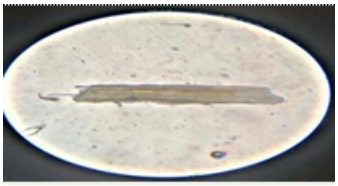

Figure 10: Vessel.

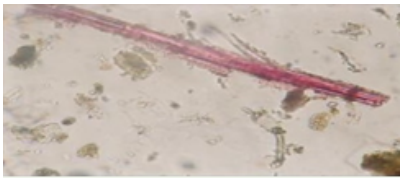

Figure 11: Lignified fiber.

Transverse section of *Ocimum sanctum (Linn.)* stem

Diagrammatic transverse section of *Ocimum sanctum (Linn.)* stem (Figures 12-15).

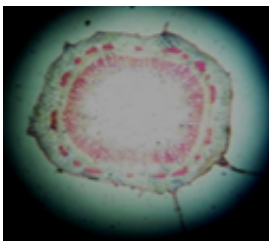

Figure 12: Diagrammatic.

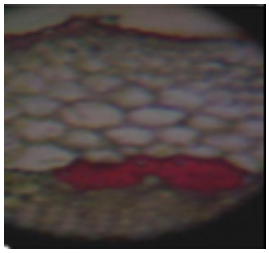

Figure 13: Outer region.

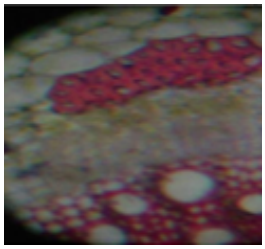

Figure 14: Middle region.

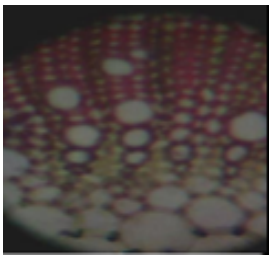

Figure 15: Inner region.

A single layer epidermis looks a like with unilateral and multicellular coating trichomes with 5-6 cells have collapsed; crust consisting of 10 or multiple layers of thin rectangular walls, parenchymal cells; phloem consists of rectangular, thin walled shielding elements cells and fibers of the parenchyma; fibers found dispersed chiefly throughout the phloem, in groups and rarely in individuals; xylem occupies most of the stem is made up of vases, tracheid fibers and parenchyma; cups without bones; fibers with pointed ends; center occupied by narrow pith consisting of round thin-walled oval parenchymal cells.

Preliminary phytochemical analysis

The results of the phytochemical studies showed that all tested extracts (aqueous, ethanol and methanol) contains the presence of alkaloids, terpenoids, flavonoids, tannins, steroids, anthroquinones, saponins, resins, glycosides and phenols. Phytochemical analysis revealed that *O. Sanctum* contained rich source of bioactive compounds such as alkaloids, terpenoids, flavonoids, tannins, steroids, anthroquinones, saponins, resins, glycosides and phenols [9-11]. Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism [12-15]. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consist of alkaloids, terpenoids and phenolic compounds. *O. Sanctum* stem extracts contained various phytochemical compounds such as saponins, alkaloids, flavonoids, cardiac glycosides, steroids, phenols and tannins [16-18].

RESILTS AND DISCUSSION

Observation table

The results are given in tabular forms (Tables 5-10 and Figures 16 and 17).

Chemical constituens	Result
Alkaloids	$+^{Ve}$
Carbohydrates	$+^{Ve}$
Tannins	$+^{Ve}$
Glycosides	$+^{Ve}$
Saponin	$+^{Ve}$
Phenol	$+^{Ve}$
Terpenoids	$+^{Ve}$
Flavanoids	$+^{Ve}$

Table 5: Phyto-chemical test.

S NO.	Extracts	%Yield
1	Aqueous	6%
2	Methanol	9%
3	Ethanol	8%

Table 6: % Yield of different extracts of *Ocimum sanctum (Linn.)* stem.

Parameter	Result
Foreign matter	2%
Moisture content	0.70%
Total ash	8.70%
Acid – insoluble ash	0.70%
Water soluble ash	3.70%

Table 7: Identity, purity and strength.

S.No	Reagents	Visible light	Short UV light (254)	Long UV light (366)
1	Powder as such	Light brown	Brown	Blackish brown
2	Powder + Nitrocellulose	Dark brown	Dark Brown	Brownish orange
3	Powder + Picric Acid	Brown	Dark Green	Dark brown
4	Powder + 1N HCL	Brownish black	Dark brown	Dark brown
5	Powder + Conc. H_2SO_4	Dark brown	Dark green	Brownish orange
6	50% H_2SO_4	Brown	Brownish black	Dark brown
7	Powder + 50% HNO_3	Yellowish brown	Black	Blackish orange

8	Powder + 1N NaOH in H_2O	Yellow	Blackish brown	Black
9	Powder + $FeCl_3$	Dark brown	Brown	Brownish violet
10	Powder + 5% KOH	Brown	Brownish black	Black
11	Powder + NH_4OH	Cocn. HCL	Pale brown	Dark brown
12	Powder + Sudan-III	Dark Brown	Blackish brown	Brown

Table 8: Fluorescence analysis of powder of *Ocimum sanctum (Linn.)* stem

S.No.	Extracts	Visible light	Short UV light (254)	Long UV light (366)
1	Aqueous	Dark brown	Brown	Blackish
2	Methanol	Dark violet	Black	Blackish green
3	Ethanol	Blackish green	Black	Black

Table 9: Fluorescence analysis of different extracts *Ocimum sanctum (Linn.)* stem.

S.No.	Extracts	Stationary Face	Solvent System	Rf value of spots
1	Ethanol extract	TLC Aluminium sheet silica gel 60F 254 plate	Toluene : Ethyl acetate : Formic acid (7:2.7:0.3)	0.36 (Yellow), 0.46 (Light yellow), 0.67 (Green), and 0.79(Yellow)
2	Methanol extract	TLC Aluminium sheet silica gel 60F 254 plate	Toluene : Ethyl acetate : Formic acid (7:2.7:0.3)	0.53 (Pink), 0.60 (Pink), 0.67(Green), and 0.80 (Pink)

Table 10: T.L.C fingerprinting profile of different extracts of *Ocimum sanctum.*

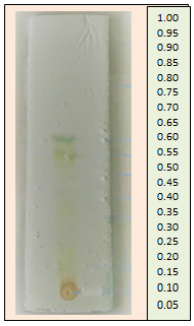

Figure 16: TLC fingerprinting profiling of ethanol extracts and Rf value of spots visualized 0.36 (yellow), 0.46 (light yellow), 0.67 (green), and 0.79(yellow).

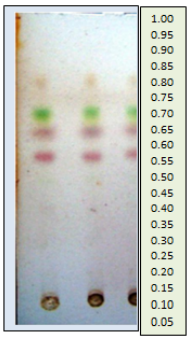

Figure 17: TLC fingerprinting profiling of methanol extract and Rf value of spots 0.53 (pink), 0.60 (pink), 0.67 (green), and 0.80 (pink).

The present review aims to document the morphology, distribution, phytochemistry and medicinal properties of *O.sanctum Linn*. stem and its future prospects for the further scientific investigation for the development of effective therapeutic compounds. Finding of this study can be employed as suitable quality control measures to ensure the quality, safety, and efficacy of this herbal drug material and also these studies may be employed as supplement information in respect of identification parameters in the way of acceptability and quality control of this plant. Now everyday phytochemical and pharmacological studies are conducted on different parts of these plants. The present literature supports the possible of *O.sanctum Linn.* as a medicinal plant.

CONCLUSION AND FUTURE PROSPECTIVE

Ocimum sanctum has various properties such as antistress, antiseptic, analgesic, anti-inflammatory, antimicrobial, immunomodulatory, hypoglycemic, hypotensive, cardioprotective and antioxidant. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituents present in *O. sanctum* have been found to be largely responsible for the therapeutic potential. This plant has various properties such as antistress, antiseptic, analgesic, anti-inflammatory, antimicrobial, immunomodulatory, hypoglycemic, hypotensive, cardioprotective and antioxidant. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituents present in *O. sanctum* have been found to be largely responsible for the therapeutic potentials. The study reveals that various secondary metabolites such as carbohydrate, tannin, flavonoids, saponins, glycoside, terpenoid, fatty acids and phenol are present in tulsi stem extract.. Stem of *Ocimum sanctum* contain water-soluble phenolic compounds and various other constituents, such as eugenol, methyl eugenol and caryophylllene that may act as an immunostimulant. Saponins act as anti-hyperlipidemic, hypotensive and cardiodepessive properties. The phytochemical constituents such as alkaloids, steroids, flavanoids, tannins, phenols and several other aromatic compounds of plants serve a defense mechanism against predation by many microorganisms, insects and other herbivore. Glycosides can act as cardio-stimulants in cases of cardiac failure. Tannins have anti-diarrheal and hemostasis properties. Flavonoids are responsible for antioxidant and immune-stimulatory properties. According to alkaloids, glycosides, flavonoids and saponins are antibiotic principles of plants and these antibiotic principles are actually the defensive mechanisms of the plants against pathogens.

ACKNOWLEDGEMENT

The authors acknowledge their profound gratitude to the Department of Pharmacy, GRD (PG) IMT Uttarakhand Technical University for providing necessary facilities and encouraging us to carry out the work. Thankful to University Grants Commission, Delhi for providing financial support (UGC/NET-JRF Fellowship) to carry out the work.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Onayade OA, Scheffer JJC, Svendsen ABJ. *Planta. Med*. **1990**; 56: 503-504.
2. Adeyemi OS, Akanji MA, Oguntoye SA. *J. Med. Plant. Res*. **2009**; 3: 420-423.
3. Dolly P. *Appl. Sci. Dept*. **2020**.
4. Pattanayak P, Behera P, Das D, Panda S.K. *Pharmacogn. Rev.* **2010**; 4(7): 95-105
5. Basil TH. Tulsi *Ocimum sanctum* Benefits, Research, Side Effects. *Ayurvedic. Herbs*. **2019**.
6. Williamson EM. *Churchhill. Liv. Stone. Pub.* **2002**; 201-205.
7. Tanwar R, Pahare A, Naqvi S. *J. Indo. Amer. Pharm. Res.* **2015**;

12(5): 2231-6876.

8. Sailaja I, Shaker IA, Ratna YK. *J. Asi. Bio. Sci*. **2010** 5(1): 1-5.
9. Bonjar GHS. Nik AK. Aghighi S. *J. Bio. Sci.* **2004**; 4: 405-412.
10. Bairwa MK, Jakhar JK, Satyanarayana Y, Reddy AD. *Sch. Res. Lib*. **2012**; 2 (3): 397-400.
11. Sood S, Narang D, Dinda AK, Maulik SK. *J. Pharm. Pharmcol*. **2005**; 57 (1): 127-133.
12. Asquith TN, Butler LG. *Phyto. Chem.* **1986**; 25 (7): 1591-1593.
13. Cowan MM. *Clin. Microbio*. **1999**; 12 (4): 564-582.
14. Khanna N, Bhatia J. *J. Ethnopharmacol*. **2003**; 88(3): 293-296.
15. WHO. Quality control methods for herbal materials, World Health Organisation. *Library. Catalog*. **1998**.
16. WHO. Quality control methods for herbal materials, World Health Organisation. *Library. Catalog*. **2011**.
17. Shastry JLN. Illustrated Dravyaguna Vijnana. *J. Pharm*. **2017**.
18. Kelm MA, Nair MG, Strasburg GM, DeWitt DL. *Phytomed*. **2000**; 7(1): 7-13.