CARAS
© 2023, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 14; Issue 03 (May-Jun 2023); pp 625–629

Open Access
ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Analyzing the Phytochemical Composition of *Justicia gendarussa* Burm F. Leaves

G. Shamili*[1] and G. Santhi[2]

[1-2] Department of Botany, Kunthavai Naacchiyaar Government Arts College for Women, Thanjavur - 613 007, (Affiliated to Bharathidasan University), Tamil Nadu, India

Abstract

Justicia gendarussa be included in the family Acanthaceae. The plant species are frequently used in research of traditional therapeutics. The macroscopy, microscopy, quantitative analysis, extractive values in ethanol and water, phytochemical screenings were estimated. Leaf is simple, entire, wavy, ovate, lanceolate, apex acuminate, 6 to 12 cm long, 2 to 5 cm wide-ranging, along with midrib major at the lower surface. T.S of midrib exposed the occurrence of a single layered epidermis, covered with cuticle and multicellular trichomes in both epidermis. The collenchymatous found near to the upper epidermis. Compactly arranged palisade parenchymatous cells with freely organize spongy parenchyma cells were observed. Endodermis layer surrounded the whole vascular bundle. Vascular bundle consists of xylem and phloem which were radically arranged. The cubical calcium oxalate crystals also observed. The physico-chemical like ash value, water and acid soluble, insoluble ash, extractive value was observed. The different extracts of *Justicia gendarussa* leaves show the existence of alkaloids, anthroquinone, coumarins, emodins, flavonoids, glycoside, polyphenol, saponin, steroids, tannin, terpenoids, triterpenoids and while anthocyanins were absent in aqueous extract, anthocyanins and emodins were absent in ethanol extract. Quantitatively flavonoid, tannin, triterpenoids and polyphenol were estimated.

***Key words*:** *Justicia gendarussa*, Macroscopic, Microscopic, Physico-chemical, Phytochemical

India has a prosperous civilizing tradition medicine. The crude drugs being always available easily in abundance, comparatively cheaper, with negligible side effects and have frequently been prescribed to patients of all age groups. The multiple therapeutic action and uses of these drugs are sufficiently described in classical literature on indigenous medicines in many medicinal plant books [18]. World Health Organization estimated over 80% of the people in increasing countries depends on traditional medicines for their primary health care [20]. India is one of the largest producers of remedial herbal as well as rightly known as a botanical garden as it is session on a good thing of well registered and traditionally well practiced familiarity of natural medicine. Nearly 17,000 species of Indian flora and 7500 species of higher plants possesses medicinal value and in other countries it is projected about7% and 13%. There are predictable to be in the region of 25,000 effective plant-based formulations, use in folk remedy known to rural community in India [22].

Look for molecules, at the minute, has taken a little different route where the science of ethno pharmacognosy is being used as guide to lead the chemistry towards different sources and classes of compounds [9]. Plant consequent natural products hold great promise for discovery and increase of new pharmaceuticals [16]. The search for in nature lively compound from pure source has always been of great benefit to researchers looking for new source of drugs useful in a variety of diseases [19]. Different genus of *Justicia* is utilized by tradition for large variety of ethno medicinal intention. *Justicia gendarussa* also known as a Nili-Nirgundi [10]. The plant grown up to one meter height found in tropical and subtropical parts of Asia and in India at seashore area. *J. gendarussa* herb is cultivated in Indian gardens for its attractive foliage and flowers [1]. Flowers of *J. gendarussa* are white through purple pink while fresh. Ethno botanically, soup of leaves of *J. gendarussa* is an admired therapy for rheumatic disease. Leaves of this plant are also used in fever, cough, jaundice, arthritis, facial paralysis and bronchitis liver disorders. A Literature survey and screening of scientific data revealed that a huge number of indigenous drugs have already been investigated as regards their botany and chemistry is concerned, however a systematic standardization is still lacking.

The present investigation includes pharmacognostical action of *J. gendarussa* it was taken to establish some botanical standards that would help in crude medicine detection also examination defilement, if any. The objective of this study macroscopic, microscopic, physico-chemical, qualitative and quantitative examinations of *Justicia gendarussa* leaf extract. The leaf is entire, simple, wavy, ovate, lanceolate, apex

Received: 10 Jan 2023; Revised accepted: 17 Apr 2023; Published online: 02 May 2023

Correspondence to: G. Shamili, Department of Botany, Kunthavai Naacchiyaar Government Arts College for Women, Thanjavur - 613 007, (Affiliated to Bharathidasan University), Tamil Nadu, India, Tel: +91 9787265232; E-mail: shamgovind2595@gmail.com

Citation: Shamili G, Santhi G. 2023. Analyzing the Phytochemical Composition of *Justicia gendarussa* Burm F. Leaves. *Res. Jr. Agril. Sci.* 14(3): 625-629.

acuminate, 6 to 12 cm long, 2 to 5 cm broad, and midrib prominent next to lower surface. A cross section of the middle of the leaf exposed the existence of single layer epidermis, covered with cuticle and multicellular trichomes in both epidermis. The collenchymatous found near to the upper epidermis. The physico-chemical constants like ash value, water and acid soluble, insoluble ash, extractive value was studied. The qualitative analysis of leaves extracts aqueous and ethanol have been reported to contain alkaloids, anthroquinone, coumarins, emodins, flavonoids, glycoside, polyphenol, saponin, steroids, tannin, terpenoids, triterpenoids and anthocyanins as a major constituent. The quantitative phytochemical analysis of *J. gendarussa* leaves was found rich in flavonoids, polyphenol, tannin and terpenoids were quantified and estimated.

MATERIALS AND METHODS

Collection of plant materials

Leaves of *Justicia gendarussa* were collected in November 2021 from Kaadukaaval, Thanjavur District, Tamil Nadu, India. The leaves were identified and authenticated RK 3008 by Botanist, Prof. Dr. S. Soosairaj, Director, The Rapinat Herbarium, St. Josephs College, Tiruchirappalli, Tamil Nadu, India.

Macroscopic and microscopic studies

Macroscopic and microscopic characters were examined [3]. Quantitative microscopy includes surface numbers like stomatal number and stomatal index were studied. Different analytical characters of fresh leaves and crushed leaves were studied by microscopic (Compound microscope) analysis with or without staining [7], [13], [14], [24].

Physico-chemical analysis

Physico-chemical parameter of the pulverized sample leaves such as ash value, extractive value and loss on drying is done as per method described in WHO guidelines [25].

Preparation of extract

Justicia gendarussa leaves were washed thoroughly in distilled water and allow to dry at room temperature at 20^{o}C in 2weeks and coarsely powdered in mortar and pestle. 10g of powder was extracted with 70% ethanol and aqueous for 24 hours.

Qualitative preliminary phytochemical analysis

Phytochemical analyses were carried out by using standard procedures [11-12], [21], [23-24].

Test for alkaloids

To a few (one) ml of the extract, a drop of Mayer's reagent is added to the side of the test tube. A smooth or fair precipitate indicates the test is positive.

Test for anthocyanins

2 ml of plant extract is additional 2 ml of 2N HCl and NH_3. Pink-red changing to blue-violet specifies the existence of anthocyanins.

Test for anthraquinones

5 ml of the extract is hydrolyzed with diluted concentrated H_2SO_4 extracted with benzene. 1 ml of diluted ammonia is added to it. Rose pink coloration recommended the positive response for anthraquinones.

Test for coumarins

3 ml of 10% NaOH was supplementary to 2 ml of plant extract formation of yellow colour point out the occurrence of coumarins.

Test for emodins

2 ml of NH_4OH and 3 ml of Benzene was added to the plant extract. Appearance of red colour indicates the occurrence of emodins.

Test for flavonoids

5 ml of diluted ammonia solution were additional to a segment of the aqueous filtrate of every plant extract follow by adding of determined H_2SO_4. A yellow colouration formed in every extract indicates the occurrence of flavonoids.

Test for glycosides (Keller-Killani test)

5ml of each extract with 2 ml of CH_3COOH contain one drop of $FeCl_3$. This is underlayed with 1ml of concentrated H_2SO_4. A brown circle of the border designates a deoxysugar feature of cardenolides. Violet rings become visible lower than the brown ring, though in the acetic acid layer, a greenish circle may form just progressively during thin layer.

Test for polyphenols

Ethanol (4 ml) is added to each extracts (1ml) and the follow-on solution is transfer in test tubes and warmed in a water bath for 15mins. 3 drops of newly prepared $[Fe(CN_6)]^{3-}$ additional to the extract solution. The Bluish green colour confirms the presence of polyphenol.

Test for saponin

About 2 ml of sample is boil in 20 ml of distilled water in a water soak and filtered. 10ml of the filtrate is assorted by 5 ml of distilled water and quake forcefully for a constant persistent froth and soak with 3 drops of olive oil and quake forcefully, after that experimental for the formation of emulsion.

Test for steriods

2ml of $C_4H_6O_3$ is supplementary to 1ml of extract of each one sample by 2 ml H_2SO_4. The colour tainted from violet into blue otherwise green in some samples indicating the presence of steroids.

Test for tannins

About 1ml of sample is heated in 20ml of water a test tube in addition to then filtered. A few drops of 0.1% ferric chloride are added and observed for brownish green or a blue-black colouration.

Test for terpenoids

5ml of both extract is mixed in 2 ml of $CHCl_3$, and concentrated H_2SO_4 (3ml) is carefully added to appearance a coating. A reddish brown colouration of the bury look is produced to prove optimistic consequences for the occurrence of terpenoids.

Test for triterpenoids

1ml of the extract is added in 1 ml of $CHCl_3$, 1 ml of $C_4H_6O_3$ is additional subsequent the addition of 2ml of Conc. H_2SO_4. Formation of glowing violet colour indicates the occurrence of triterpenoids.

Quantitative analysis

Total phenols evaluated by the process [5], flavonoid set on by the process of [2], total terpenoid substance by standard method [6], Tannin was followed by [4] method.

Determination of flavonoid

10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125mm). The filtrate was later on evaporated into drought over a water soak in addition to weighed to a stable weight.

Determination of total phenols

Plant powder (2g) is heat with 50ml of ether for that extraction of the phenolic element for 15min. 5ml the extract was drained a through tube into 50ml flask, then 10ml of distilled water has been added. 2ml of NH_4OH solution and 5ml of strenuous $C_5H_{12}O$ added. The samples were made up left to respond for 30min for colour development. This was measured at 505nm.

Determination of tannin

500mg of sample weighed into a 50ml plastic bottle. 50ml of distilled water was add and shake in to 1hr in a motorized shaker and filtered through 50ml volumetric flask and make up in the mark. 5ml filtrate was pipetted into experiment tube in addition to assorted with 2ml of 0.1M $FeCl_3$ in 0.1N hydrogen chloride and 0.008M $C_6FeK_4N_6$. The absorbance was measured at 120nm within 10min.

Estimation of total terpenoid content

1 g of sample was taken separately and soaked in alcohol (50ml) for 24 hrs. Then filtered, the filtrate was extract with C_6H_{14} (40ml) for 2 hours. The dried ether extract was evaporated by complete elimination of petroleum ether under reduced pressure. The dried ether extract was treated as total terpenoid.

RESULTS AND DISCUSSION

Macroscopic characterization

Justicia gendarussa is a Shrub, branches gloomy lavender, terete, smooth. Leaves 7-10 × 2cm, linear or oblong-lanceolate, apex acute, base acute, chartaceous, glabrous, lateral nerves 5-7 pairs, bluish; petiole 2-3mm long. Spikes terminal, to 8cm long, narrow; bracts linear, 4mm long. Flowers are white, calyx lobes linear-lanceolate, 5mm long; corolla white with purple streaks, 1.5cm long; ovary and style puberulus. Capsule 12mm long, glabrous, characteristic odour, bitter in taste. Leaf has short stalk *J. gendarussa* has small white flowers arranged in the form closed grain, spread and out of the tip of the stalk show in (Fig 1-2, Table 1).

Table 1 *Justicia gendarussa* leaf macroscopic characterization

S. No.	Characters	Observation
1	Colour	Dark green
2	Odour	Characteristic odour
3	Taste	Bitter
4	Length	12 cm
5	Margin	Smooth margin (entire)
6	Apex	Sharp (acuminate)
7	Base	Acute
8	Surface	Smooth surface
9	Shape	Lanceolate
10	Vein	Pinnate
11	Stipules	No
12	Leaflets	Two pairs
13	Main nerves	8-10 pairs
14	Petioles	7 cm long
15	Width	2-5 cm

Fig 1 Habit of *Justicia gendarussa*

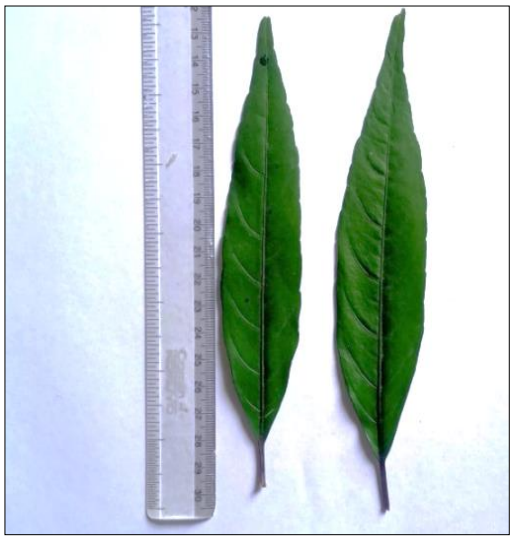
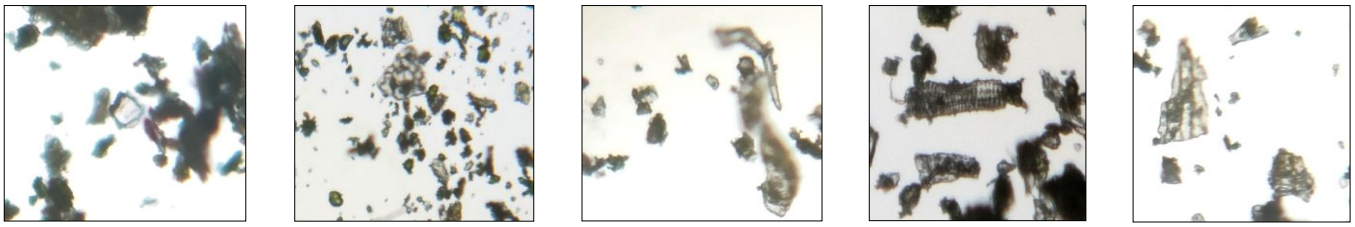


Fig 2 Morphology of *Justicia gendarussa* leaf

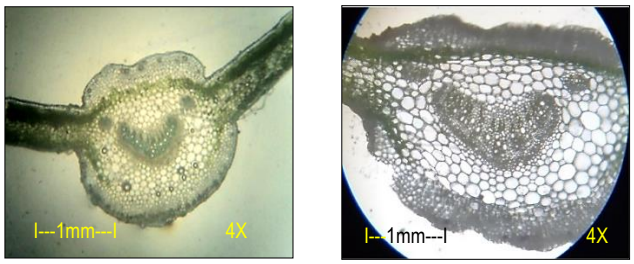
Microscopic characterization

T.S of midrib shows a planoconvex outline in the basal and biconvex in middle and apical regions. It shows a covered epidermis covered externally with cuticle and presence of both covering (uniserriate and unicellular) and glandular trichomes (having unicellular stalk and multicellular head). Collenchyma is well developed, which is present bellow the upper epidermis and above the lower epidermis. In the middle region, there are three vascular bundles, which are collateral and open. The central one is large and arc shaped. T.S of lamina shows single layer of epidermis followed by two ayered palisade parenchyma cells, compactly arranged columnar cells filled with chlorophyll. Mesophyll cells are made up of ovate spherical parenchyma cells. They are loosely set with intercellular spaces. Cubical calcium oxalate crystals present in upper epidermis. Anisocytic stomata were observed in the lower epidermis in (Fig 3-4).

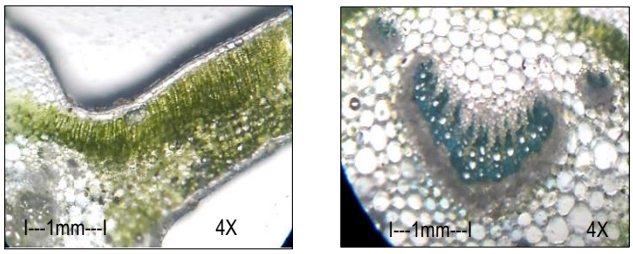


a) Calcium oxalate crystals b) Parenchyma cells c) Tricomes d) Palisade tissue e) Xylem vessels

Fig 3 *Justicia gendarussa* leaf microscopic characterization



a) Calcium oxalate crystals b) Parenchyma cells



d) Palisade tissue e) Xylem vessels

Fig 4 T.S of *Justicia gendarussa* leaves

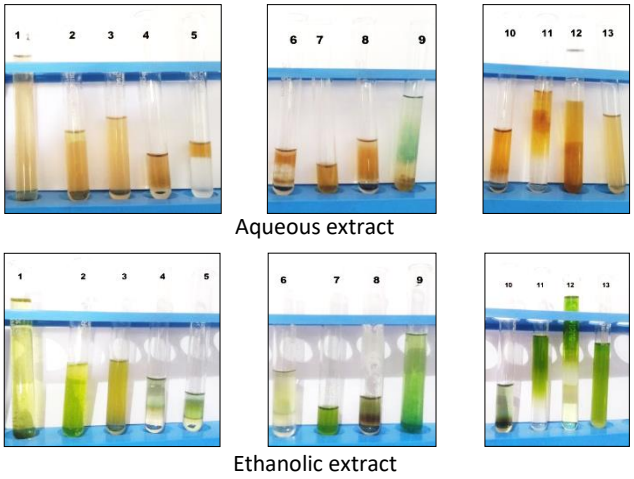


Fig 4 Phytochemicals qualitative investigation of *Justicia gendarussa* leaves aqueous and ethanol extracts

1. Tannin, 2. Saponnin, 3. Flavonoids, 4. Steroids, 5.Terpenoids, 6. Triterpenoids, 7. Alkaloids, 8. Anthroquinone, 9. Polyphenol, 10. Glycoside, 11. Coumarins, 12. Emodins and 13. Anthocyanins

Physico-chemical analysis

The physico-chemical constants like ash value, water and acid soluble, insoluble ash, extractive value was studied. The loss on drying of *J. gendarussa* at 105ºC in 12.56±0.86% recorded respectively. The dried leaf powder of *J. gendarussa* shows higher percentage total ash (14.32±0.98%), acid insoluble ash (1.20±0.08%), water soluble ash (5.32±0.37%), sulphated ash (3.5±0.255) alcohol soluble extractive (12.62±0.88%) and water-soluble extractive values (18.53±1.26%) recorded and presented in (Table 2).

Table 2 Shows the physico-chemical analysis *Justicia gendarussa* leaves powder

S. No.	Tests	As per analysis (%)
1	Loss on drying at 105 ºC	12.56 ± 0.86
2	Total ash	14.32 ± 0.98
3	Acid insoluble ash	1.20 ± 0.08
4	Water soluble ash	5.32 ± 0.37
5	Sulphated ash	3.5 ±0.25
6	Alcohol soluble extractive	12.62 ±0.88
7	Water soluble extractive	18.53 ±1.26

Values expressed as Mean ± SD for triplicates

Phytochemical qualitative analysis of J. gendarussa leaves

The qualitative analysis of the leaves of *J. gendarussa* present in (Table 3, Fig 4). The phytochemical screening reveals the aqueous and ethanol extract of *J. gendarussa* leaves show the existence of alkaloids, anthroquinone, coumarins, emodins, flavonoids, glycoside, polyphenol, saponin, steroids, tannin, terpenoids, triterpenoids and anthocyanins were absent in aqueous extract, anthocyanins and emodins were absent in ethanol extract.

Table 3 Qualitative analysis of *Justicia gendarussa* leaves extracts

S. No.	Phytochemicals	Extract	
		Aqueous	Ethanol
1	Alkaloids	+	+
2	Anthocyanins	-	-
3	Anthroquinone	+	++
4	Coumarins	++	+
5	Emodins	++	-
6	Flavonoids	+	++
7	Glycoside	++	++
8	Polyphenol	++	++
9	Saponin	++	++
10	Steroids	+	++
11	Tannin	+	++
12	Terpenoids	++	++
13	Triterpenoids	++	++

(-) Absent, (+) Present and (++) high concentration

Phytochemical quantitative analysis of J. gendarussa leaves

The quantitative phytochemical investigation of *J. gendarussa* leaves was found rich in flavonoids (98.57±6.86mg/gm), polyphenol (143.67±10.05mg/gm), tannin (68.45±4.76mg/gm) and terpenoids (56.80±3.92mg/gm) were recorded and presented in (Table 4).

Table 4 Quantitative analysis *Justicia gendarussa* leaves

S. No.	Phytochemicals	Results (mg/gm)
1	Flavonoids	98.57±6.86
2	Polyphenol	143.67±10.05
3	Tannin	68.45±4.76
4	Terpenoids	56.80±3.92

Values expressed as Mean ± SD for triplicates

The study provides information in respect to their identification, chemical constituents and physico-chemical characters which may be useful for pharmacognostical study and standardization of herbal drugs of traditional medicine. It will also determine therapeutic diagnostic tools for the scientists who are keen and sincere to evaluate the herbal medicine of indigenous resources. Morphological identity of leaf of *J. gendarussa* has midrib and major nerves have prominent violet and bilabiate flower which is characteristics of the Acanthaceae family similar work to [18]. Previous [17] reported presence of starch grains in the endodermis and cystolith the identify the leaves of dicotyledons. The present microscopically studies show the presence of cystolith, diacytic stomata, trichomes and sessile glanduler trichome supports the fact that, this plant belongs to Acanthaceae family. Identifying character of leaf is presence of cystolith in epidermis and starch grains in endodermis and lamina. Acid insoluble ash was lower since the presence of cystolith in large number. Similarly [8] reported the physico-chemical studies of *Justicia adhotoda* L. total ash (20%), foreign matter (20%), acid insoluble ash (82%), water soluble ash (4.5%), alcohol soluble extractive (6.8%), water soluble extractive (18.45%) and sulphated ash were present. The present investigation also similar to the physico-chemical constants like ash value, water and acid soluble, insoluble ash, extractive value. Whereas higher percentage of total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive and the sulphated ash were recorded.

In the current investigation aqueous extract was found in highest quantity of polyphenols flavonoids and terpenoids present in *Justicia gendarussa* leaves. Phytochemical screening indicated that the leaf of *Justicia gendarussa* was rich in phenolics, alkaloids, flavonoids, and triterpenoides constituents. Related to [15] reported that the preliminary phytochemical screening of *Justicia tranquebariensis* L. showed the presence of plant components such as carbohydrates, flavonoids, quinones and coumarins in hexane extract, carbohydrates, tannins, flavonoids, cardiac glycosides, terpenoids, phenols, coumarins and steroids in ethyl acetate extract and carbohydrates, tannins, flavonoids, quinones, cardiac glycosides, phenols and coumarins in ethanol extract. Secondary metabolites which are responsible for therapeutics of the plant are the characteristic feature of the family of Acanthaceae.

CONCLUSION

Therefore, the work on pharmacognostical characteristics and phytochemical screening of *Justicia gendarussa* provide useful information, which may help in authenticating the genuine plant along with nature of phytoconstituents present in it. Present study is the first-time report of standardization of *Justicia gendarussa* leaf. The plant contains many secondary metabolites. Hence proper isolation of the active principles might help in the findings of new lead compounds. This established a significant scope to develop a broad spectrum used in *Justicia gendarussa* herbal medicine and as a base for the development of novel potent drugs.

LITERATURE CITED

1. Bendre A, Kumar A. 1996. *A Text Book of Practical Botany*. 6th Edition, Rastogi Publication. 2: 1621.
2. Boham BA, Kocipai-Abyazan R. 1974. Flavonoids and condensed tannins from leaves of *Vaccinium vaticulatum* and *V. calycinium*. *Journal of Pacific Science* 48: 458-463.
3. Brain KR, Turner TD. 1975. *The Practical Evaluation of Phytopharmaceuticals*, Wright-Scientechnica. *Bristol*. 4-9: 36.
4. Burden -Van TP, Robinson WC. 1981. Formation of complexes between protein and Tannic acid. *Journal of Agricultural Food Chemistry* 1: 77.
5. Edeoga HO, Okwu DE, Mbaebie BO. 2005. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4(7): 685-688.
6. Ferguson NM. 1956. *A Text Book of Pharmacognosy*. Mac Milan Company, New Delhi. pp 191.
7. Goyal RK, Shah BS. 2005. *Practical in Pharmacognosy*. Nirali Prakashan, Pune. pp 128-155.
8. Gupta A, Joshi A, Joshi VK. 2014. Pharmacognostical study of *Justicia adhatoda* Linn. leaf. *International Journal of Herbal Medicine* 1(6): 01-04.
9. Gurib FA. 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mol. Aspects Med.* 27(1): 1-93.
10. Handa SS, Sharma A, Chakraborti KK. 1986. Evaluation of Indian herbal hepatoprotective drugs. *Fitoterapia* 57(5): 307-351.
11. Harborne JB. 1973. *Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis*. 2nd Edition, London New York.
12. Harborne JB. 1984. *Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis*. 2nd Edition, London New York.
13. Jain VK, Jain B, Saha D. 2011. Pharmacognostical and physico-chemical evaluation of *Pterospermum suberifolium* Linn leaves, *Int. Jr. Pharma. Res. Develop*. 2(11): 89-104.
14. Kokate CK 1986. *Practical Pharmacognosy*. 1st Edition. Vallabh Prakashan, New Delhi. pp 111.
15. Krishnamoorthi R, Ratha Bai V. 2015. Phytochemical analysis and antioxidant property of *Justicia tranquebariensis* R. *International Journal of Pharmacognosy* 2(5): 254-258.
16. McChesney JD, Venkataraman SK, Henri JT. 2007. Plant natural products: back to the future or into extinction? *Phytochemistry* 68(14): 2015-2022. doi: 10.1016/j.phytochem.2007.04.032.
17. Metcalfe CR, Chalk L. 1950. *Anatomy of the Dicotyledons*. Vol. 1, Clarendon Press, Oxford. pp 243-245.
18. Patel S, Kapadia N, Shah B, Shah M. 2011. Botanical identification and physico-chemical investigation of leaf of *Nili-nirgundi* (*Justicia gendarussa*). *International Journal of Pharmaceutical Sciences Review and Research* 10(1): 116-121.
19. Shamili G, Santhi G. 2019. Identification and characterization of bioactive compounds of leaves of *Justicia Gendarussa* Burm. F. *International Journal of Scientific Research in Biological Sciences* 6(1): 145-153.
20. Shankar D, Majumdar B. 1993. Beyond the biodiversity convention: The challenges facing the biocultural heritage of India's medicinal plants. *In*: Medicinal plants for forest conservation and health care. *Non-wood Forest Products Services* 11: 163.
21. Sofowara A. 1993. *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Ltd, Ibadan, Nigeria. pp 289.
22. Verma S. 2016. Medicinal plants used in cure of skin diseases. *Advances in Applied Science Research* 7(3): 65-67.
23. Trease GE, Evans WC. 2002. *Pharmacognosy*. 15th Edition. E. Dinburg, London. 475: 101-105.
24. Trease GE, Evans WC. 1989. *Pharmacognsy*: 11th Edition. Brailliar Tiridel Can. Macmillian Publishers.
25. WHO. 1998. *Quality Control Methods for Medicinal Plant Materials*. Word Health Organization, Geneva.