JOPAT Vol 23(2), 1398- 1410, July – December, 2024 Edition.
ISSN2636 – 5448 https://dx.doi.org/10.4314/jopat.v23i2.1

Pharmacognostic Standardisation and Antioxidant Activity of *Cassia sieberiana* DC. (Fabaceae)

Mubo Adeola Sonibare [1*], Caleb O. Opone[1,2], Akingbolabo Daniel Ogunlakin[3*], Opeyemi Josephine Akinmurele[4], Samuel Abayomi Adebodun[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria. sonibaredeola@gmail.com; yadebodun@gmail.com

[2]Jaja Clinic, University Health Services, University of Ibadan, Ibadan, Oyo State, Nigeria. calebcr7.co@gmail.com

[3]Phytomedicine, Molecular Toxicology, and Computational Biochemistry Research Laboratory (PMTCB-RL), Department of Biochemistry, Bowen University, Iwo 232101, Nigeria. gbolaogunlakin@gmail.com

[4]Department of Pharmacognosy, Faculty of Pharmacy, Lead City University, Ibadan, Nigeria. opeyemiakinmurele@gmail.com

ABSTRACT

Secondary metabolites of medicinal plants can be used as dietary antioxidants, acting as free radical scavengers, radical chain reaction inhibitors, metal chelators, oxidative enzyme inhibitors and antioxidant enzyme cofactors. However, the major setback in promoting the use of medicinal plants is the lack of standardisation. This study aimed to set pharmacognostic standards and establish the antioxidant activity of *Cassia sieberiana* via *in vitro* method. *Cassia sieberiana* leaves methanol extract was partitioned into *n-hexane*, DCM, and ethyl acetate fractions. The macroscopic, microscopic and chemo-microscopic characters were assessed while antioxidant potential of the crude extract and partitioned fractions was assessed using DPPH radical scavenging assay. Total phenolic and total flavonoid contents were evaluated using gallic acid and quercetin as standards. The transverse section showed presence of bundle sheath and the parenchyma cells. The adaxial and abaxial surfaces showed straight polygonal epidermal cells and presence of palisade cells. Also, the adaxial surface showed the presence of tector trichomes while the abaxial surface showed presence of glandular trichomes. The ethyl acetate fraction had the highest antioxidant activity ($IC_{50} = 71.42 \pm 0.19$ µg/mL), the highest total flavonoid content (TFC) and total phenolic content (TPC) with 203.19 ± 0.03 mgQE/g and 25.10 ± 0.61 mg GAE/g, respectively. Standards have been set in this study for the identification and authentication of *Cassia sieberiana*, and the *in vivo* antioxidant activity of the plant supported the traditional use of the plant for managing different ailments, which may enhance the production of reactive oxygen in the body system.

Keywords: *Cassia sieberiana*, Standardisation, Microscopy, Phyto-constituents, Antioxidant.

*Corresponding authors: sonibaredeola@gmail.com; gbolaogunlakin@gmail.com

INTRODUCTION

Historically, medicinal plants have been utilized as an important source of biologically active chemicals for the production and development of novel medications in practically every culture [1, 2]. Eighty percent of the world's population is reported to rely on herbal remedies and use them in primary healthcare [3]. Still, only a tiny percentage of the estimated plant species globally have undergone phytochemical research, despite the widespread usage of medicinal herbs [4]. The process of choosing and managing raw materials, assessing the stability, safety, and effectiveness of the final product, and recording risks and safety information are all part of the standardization process [5, 6]. If an herbal product has not been verified and described to guarantee consistency in its production, it cannot be regarded as scientifically valid [7]. The quality indices used in standardization are chromatographic analysis, moisture content, ash values, extractive values, foreign organic matter, macroscopic and microscopic evaluation, as well as quantitative and qualitative chemical examinations [8].

©2024 The authors. This work is licensed under the Creative Attribution 4.0 International license.

Oxidative stress occurs when free radicals build up naturally because of metabolic processes. In the pathophysiology of chronic illnesses like diabetes, cancer, rheumatoid arthritis, neurological diseases, and cardiovascular diseases, oxidative stress is a crucial factor [9, 10]. Antioxidants are essential for their ability to scavenge free radicals and active oxygen species. Greater resistance to several kinds of diseases is correlated with higher levels of antioxidants [11, 12]. Plants are a reasonable source of antioxidants; this is especially true of plants with high phenolic and flavonoid contents 13. Since oxidative stress appears to be one of the causes of many human diseases, medicinal herbs having antioxidant qualities can be used to manage or prevent many conditions [14, 15].

Cassia sieberiana, also known as *Cassia kotschyana* Oliv, is a member of the Fabaceae family. "Drum stick tree" or "West African Laburnum" is its colloquial name [16]. *Cassia sieberiana* is between 10 and 20 meters tall. The bark has shades ranging from black to dark grey. The leaves are grouped into leaflets, which have seven to ten opposite pairs of leaves. From February to March, the dry season, the blossoms are a vivid golden color. The green sepals measure 6-7 mm in length, but the petals measure 15-20 cm. In West and Central Africa, *Cassia sieberiana* is widely distributed in the Sudan savannah and the southern Sahel [17]. The pharmacological characteristics of *C. sieberiana* species include laxative, mucosal relaxant, antispasmodic, anti-inflammatory, antinociceptive, and antioxidant [18]. Tannins and astringents found in the root and stem along with glycosides, saponins, and steroids in the root and seeds, are the primary sources of the plant's numerous therapeutic applications. The roots are used as a diuretic and vermifuge to cure a variety of illnesses, including hemorrhoids, dysentery, elephantiasis, leprosy, diarrhea, and venereal diseases [19]. Numerous chemicals that are bioactive in *Cassia sieberiana* have been identified via studies on the plant. Since the ethanolic extracts of *Cassia sieberiana*'s root and stem bark have been shown to have anti-plasmodial activity against mice, they may be investigated for the treatment of malaria [20]. Nevertheless, a report on *C. sieberiana*'s standardization is not available despite all the research on this species. Moreover, there is no information available regarding *C. sieberiana* leaves antioxidant activity. Hence, the aim of this study is to standardize *Cassia sieberiana* and to evaluate the antioxidant activity of its leaves.

MATERIALS AND METHODS

Collection of plant material

The fresh sample of *Cassia sieberiana* leaves used in this project work was collected in April 2017 at the Botanical Garden, Ibadan, and authenticated at the Forestry Research Institute of Nigeria with FHI no: 109803.

Preparation of plant material

Collected fresh leaves of *Cassia sieberiana* were air dried for 15 days and powdered using a blender. The air-dried and powdered leaves of *C. sieberiana* (670 g) was macerated in a glass jar with 4 L of methanol for a period of 72 h at room temperature [21]. This was stirred occasionally with a glass rod and filtered. The residue was macerated again with another 4 L of methanol for another 24 h, stirred with a glass rod, and filtered. The residue was macerated for the third time and the procedure was repeated as above. The filtrates were combined and then concentrated using a rotary evaporator. The extract was used for qualitative and quantitative estimation of phytochemicals and determination of antioxidant activity.

Pharmacognostic characterization

Organoleptic evaluation and powder microscopy

The odor, taste, color, and texture of the powdered material were observed and recorded [22]. Microscopic examination was conducted using powdered samples, as well as transverse and longitudinal sections of the leaves of *Cassia sieberiana*. Both quantitative and qualitative studies were carried out on the leaves using standard methods [23]. The leaf powder of *Cassia sieberiana* was cleared using 2 % sodium hypochlorite and stained using safranin O before mounting of small portion on a clean slide with the addition of dilute glycerol and observed under the compound microscope for the presence of cell inclusions such as cellulose, starch, fat and oil, tannins, and calcium oxalate crystals.

Fluorescence analysis

A small quantity of powdered sample of *Casia sieberiana* was treated with 50 % sulphuric acid, hydrochloric acid, nitric acid, and picric acid. Observations were made under visible

light and UV light of short (254 nm) and long wavelength (365 nm) separately [24].

Physico-chemical analysis

Coarse powder of the plant leaves was used to assess physicochemical parameters such as alcohol and water-soluble extractive values, moisture content, total ash, and acid-insoluble ash [25].

Phytochemical screening

Phytochemical screening was carried out on the powdered leaves extracted in various solvents to detect the presence or absence of phytochemicals such as glycosides, saponin, sterols, anthraquinones, and alkaloids in the sample using standard procedures [26-28].

Measurement of total phenolic content (TPC)

The Folin-Ciocalteu method was used in measuring the total phenolic content following standard procedure [29]. In this method, 5 mL of Folin-Ciocalteu reagent (diluted tenfold) was introduced into 1 mL of each aliquot of the test sample (100 µg/mL) and was allowed to stand for 3 minutes after which 4 mL of 7.5% Na_2CO_3 solution in distilled water was added to the mixture and the procedure prepared in triplicates. The mixture content was thoroughly mixed and incubated at 27°C for 30 minutes. One milliliter (1 mL) methanol, 5 mL of Folin Ciocalteu reagent, and 4 mL of 7.5% served as the blank. Using a UV-VIS spectrophotometer, the absorbance of the mixture after 30 minutes of incubation was read at 765 nm. A linear dose-response regression curve was generated using an absorbance reading of gallic acid (12.5-0.39 µg/mL) at the wavelength of 765 nm. The result of TPC was expressed as mg GAE/g of dry weight of extracts and the TPC in the plant extract was calculated using the formula below:

$$\mathrm{TPC} = \left[\frac{\mathrm{C}\ x\ \mathrm{V}}{\mathrm{M}}\right]$$

Where TPC is the total phenolic contents of the dry weight of extracts (mg GAE/g), C is the concentration of equivalent gallic acid derived from the calibration curve (µg/mL), V is the volume of extract (mL), and M is the weight of plant extract (g).

Measurement of total flavonoid content (TFC)

The aluminum chloride colorimetric method was used for this analysis in which quercetin was used as a standard for the calibration curve and following standard procedure [29]. In this procedure, ten milligrams of quercetin were dissolved in ethanol and then diluted in the range of 100-6.25 µg/mL. The diluted standard was separately mixed with 1.0 mL of ethanol, 0.1 mL of 1% aluminum chloride, 0.1 mL of IM potassium acetate, and 2.8 mL, and distilled water. In the same way, ten milligrams of the test sample were dissolved in 10 mL ethanol and 1 mL of each together mixed with the reagents used above in the standard were mixed thoroughly in a test tube. The amount of 1% aluminum chloride was substituted by the same amount of distilled water in the blank. Incubation was done at 27°C for 30 minutes and the absorbance of the reaction was measured at 415 nm using a UV-VIS spectrophotometer. A yellow color indicates the presence of flavonoids. This assay was carried out in triplicate. The concentration of flavonoids was read from the calibration curve based on the measured absorbance and the content of the flavonoid in the extract was expressed in terms of quercetin (mg quercetin/g of extract).

Antioxidant activity (DPPH Radical Scavenging Assay)

Radical scavenging activity was carried out using standard procedure [29]. Methanol solution (1 mL) of the test sample and standard (Gallic acid) were mixed separately with 3 mL (0.004 %) of freshly prepared 1,1-diphenyl-2-picryl-hydrazyl-hydrate (DPPH) at different concentrations (200, 100, 50, 25, 125, 625, and 3.125 µg/mL). In the control, 1 mL methanol replaced the test sample. The reaction mixtures were incubated at 27°C and allowed to react for 30 minutes in the dark. After 30 minutes, the absorbance was measured at 517 nm using a UV-VIS spectrophotometer which was then converted to the percentage of antioxidant activity. The results were recorded in triplicate and the concentration required to scavenge 50% of the DPPH free radical (IC_{50}) was determined. The percentage of inhibition of DPPH (%) was calculated as follows:

$$\%\ \mathrm{Inhibition} = \left[\frac{\mathrm{Absorbance\ of\ control} - \mathrm{Absorbance\ of\ test\ sample}}{\mathrm{Absorbance\ of\ control}}\right] x\ 100$$

Statistical analysis

Data were presented as mean ± standard deviation (SD) of three separate experiments.

One-way analysis of variance was used to analyze the data.

RESULTS

Table 1 summarizes the plant's overall vegetative macro-morphological characteristics. Both the base and apex of the leaf have a complete margin and are sharp. The leaf has a glossy, leathery surface, a straightforward shape, and a shiny texture. It is green in color. Petioles are present, and the arrangement of the leaves on the petiole and stem is opposite, with deciduous stipules. The typical leaf measures 5.4 cm in breadth and 5.6 cm in length. Table 2 presented the organoleptic properties of powdered leaves of *Cassia sieberiana*. The texture is coarse with an army green color. It also has a bland taste with a characteristic odour. The components contained in the powdered plant sample were analyzed chemically using phytochemical and fluorescence analysis (Table 3). The analysis of powdered *Cassia sieberiana* leaves yielded a moisture content of 8.9%, which is within the acceptable range (Table 4). Table 5 displays the plant's powdered sample extractive value and percentage yield in ethanol and water. The chemo-microscopic characteristics of the powdered *C. sieberiana* leaves were compiled in Table 6, which also revealed the presence of tannin, starch, oil, lignin, and calcium oxalate crystals. A qualitative microscopic examination revealed stomata, trichomes, polygonal epidermal cells, and palisade cells (Table 7), while the adaxial surface showed uniseriate, unicellular tector trichomes, and abaxial surface showed sparsely distributed glandular trichomes (Figures 1 and 2). Figure 3 presents the presence of anisocytic stoma on the adaxial surface (Figure 3). The transverse section of *Cassia sieberiana* petiole revealed palisade cell, vascular bundle sheet, cortex, phloem, xylem and starch granule, while the longitudinal section features of *C. sieberiana* showed tector trichome, epidermal cell, leaf primordium and tunica (Figures 4 and 5). Glycosides, flavonoids and flavonoids, saponins, phenols and tannins, coumarins, alkaloids, and anthraquinone glycosides were found in *C. sieberiana* leaves (Table 8). Table 9 presents the antioxidant potentials, TPC, and TFC of the solvent fractions and crude extract of *C. sieberiana*.

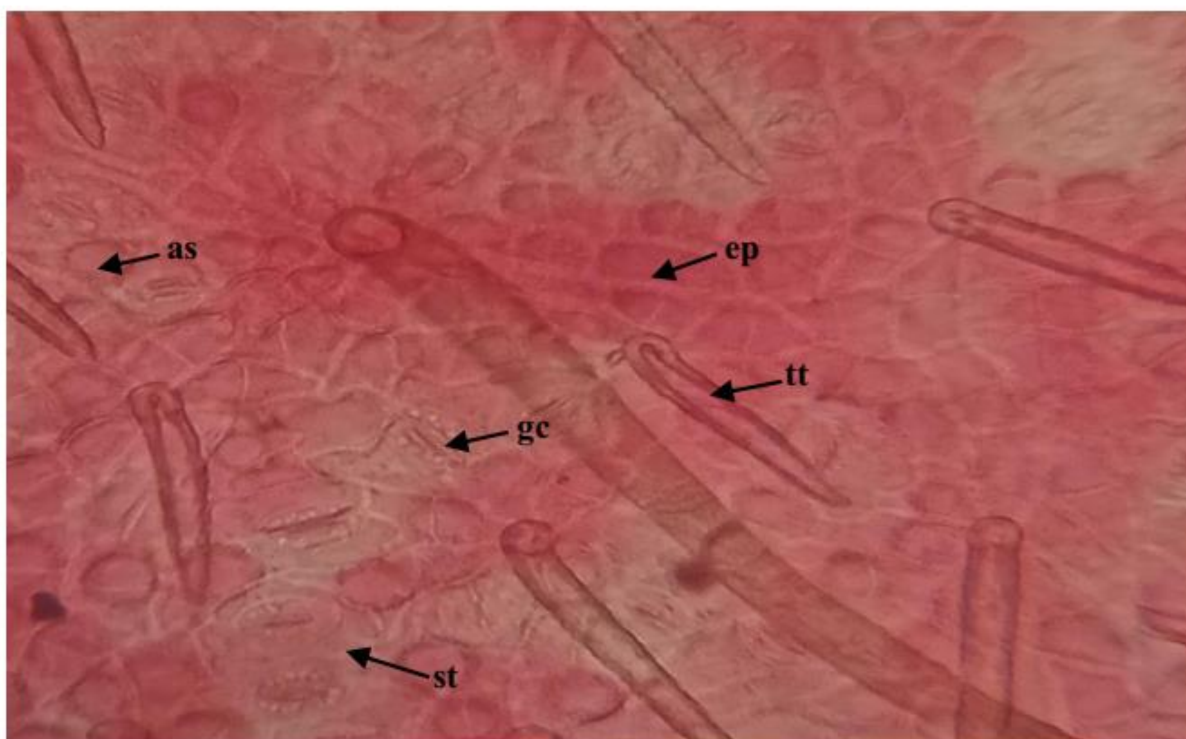

Figure 1: Adaxial *feature of C. seberina leaf showing* as = anisocytic stoma, ep = epidermis cell, gc = guard cell, tt = tector trichrome, st = stomata (Magnification: x 400)

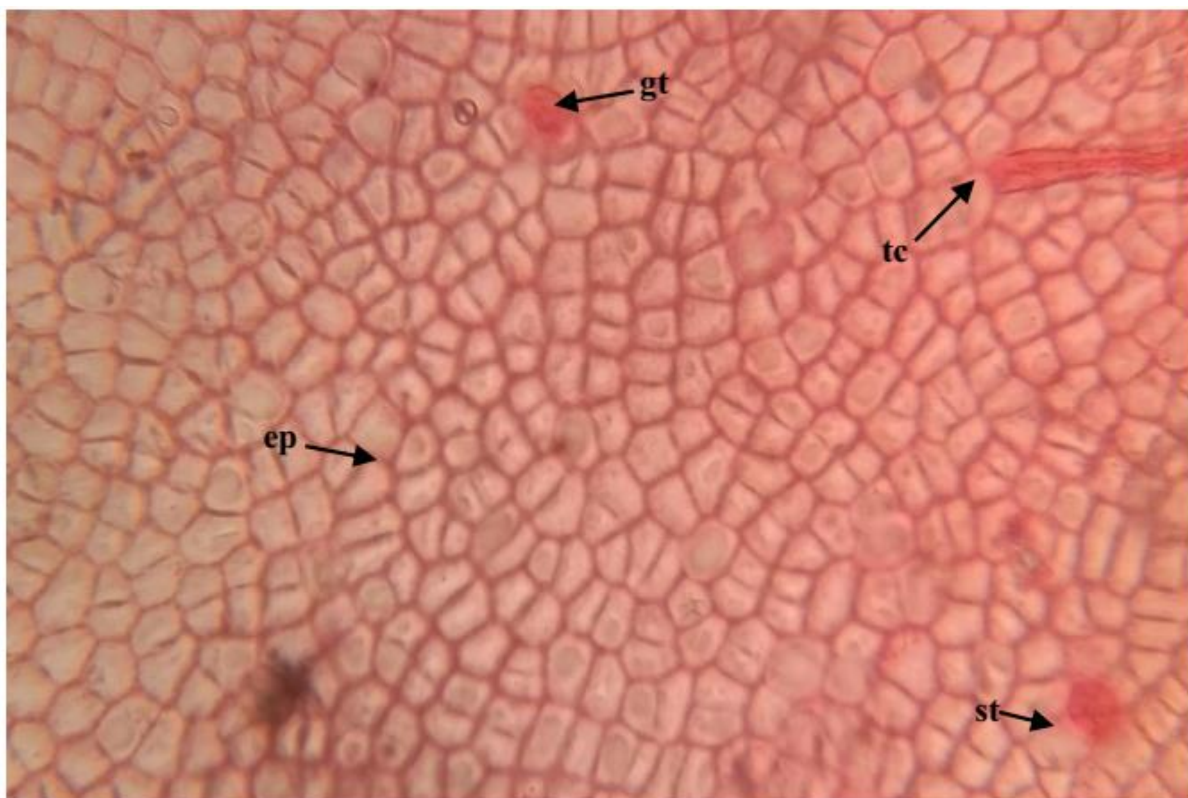

Figure 2: Abaxial *feature of C. seberina showing* ep = epidermis cell, gt = glandular trichome, tc = trichrome, st = stomata (magnification of x 400)

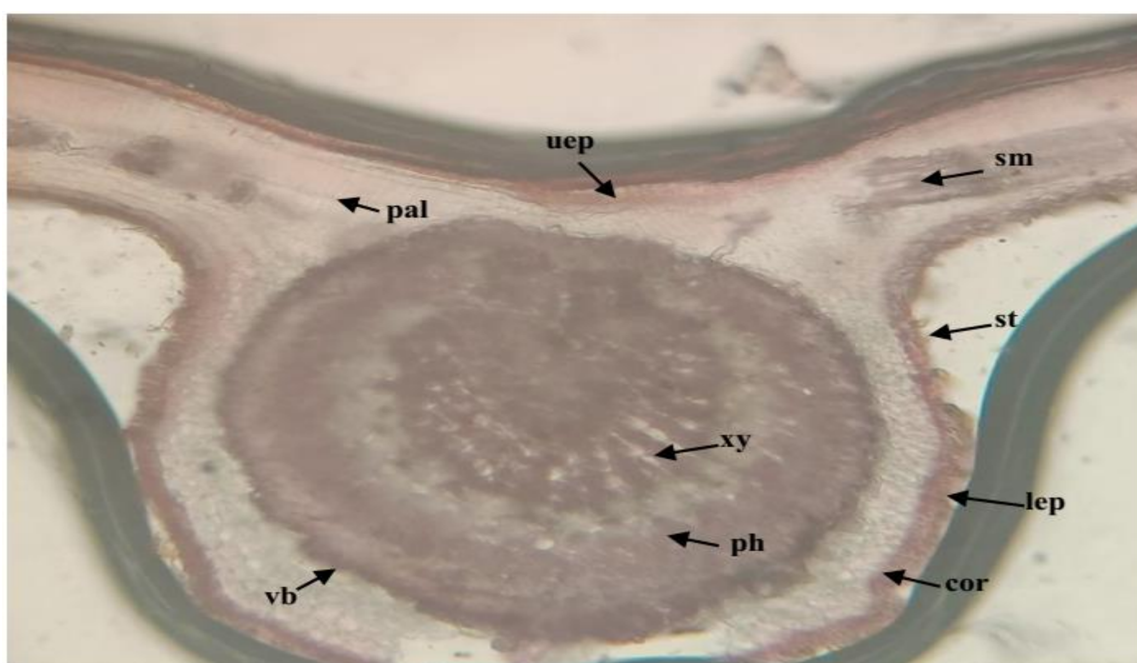

Figure 3: Transverse section *feature of C. seberina petiole showing the following:* uep = Upper epidermis, pal =Palisade cell, sm= spongy mesophyll, vb = vascular bundle sheet, xy = xylem, Ph = phloem, cor = cortex, lep= Lower epidermis, st = Stomata (Mag: x100)

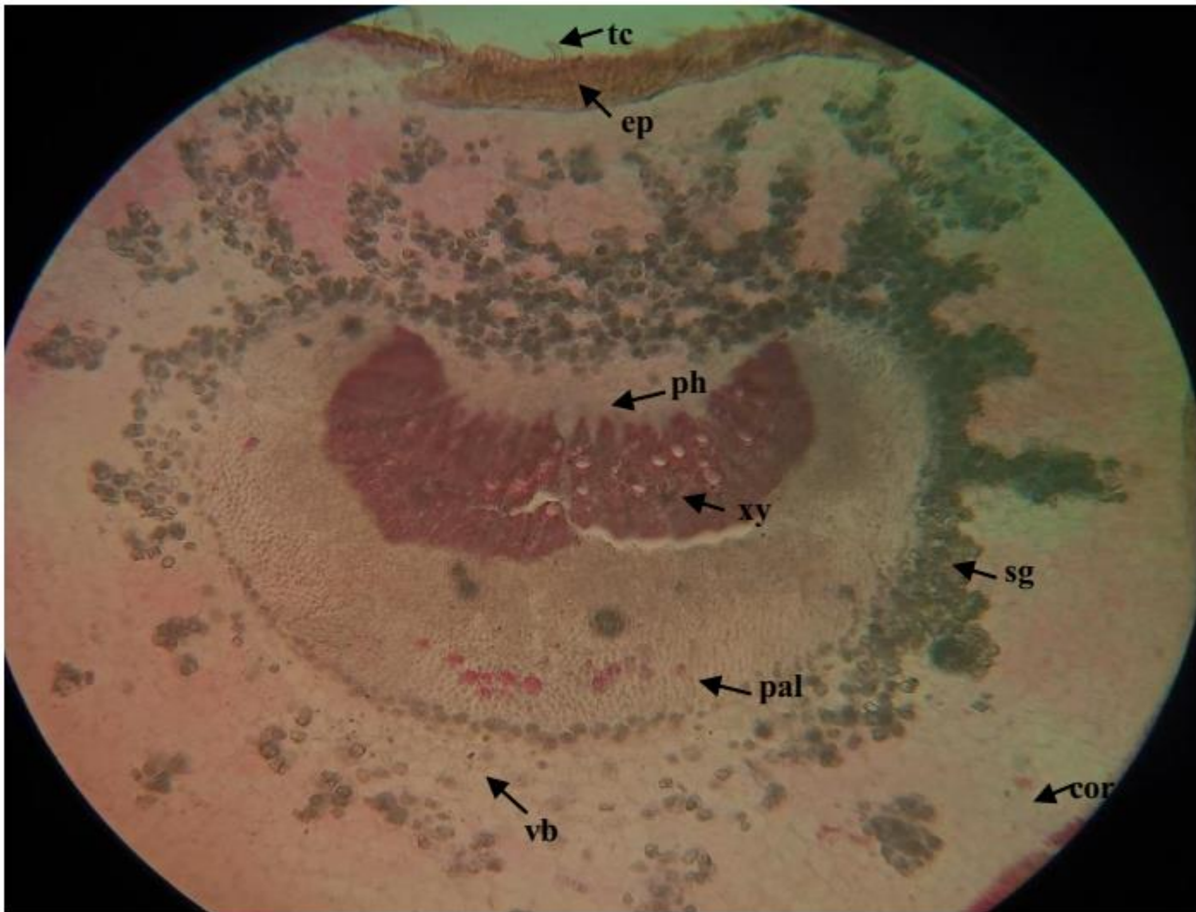

Figure 4: Petiole feature of *C. seberina showing* tc = trichrome, ep= epidermal cell, ph = phloem, xy = xylem, sg = starch granule, pal = palisade cell, vb = vascular bundle, cor = cortex (Mag: x100)

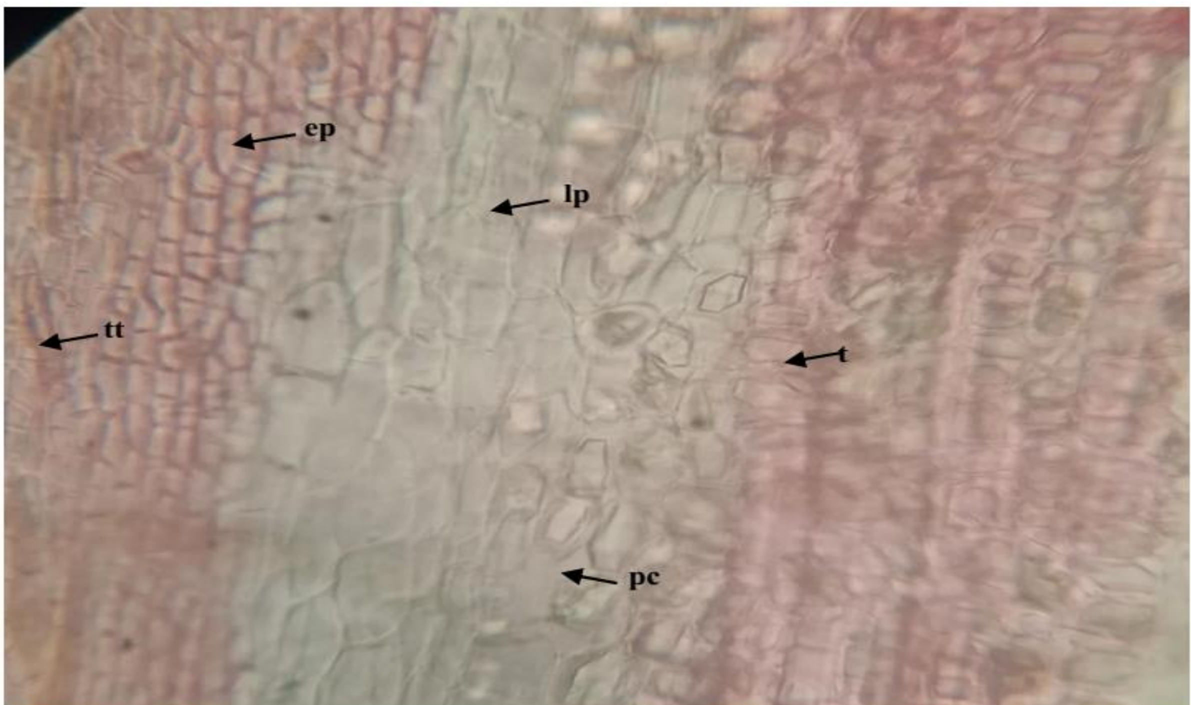

Figure 5: Longitudinal section *feature of C. seberina showing* tt = tector trichrome, ep = epidermal cell, lp = leaf primordium, pc = procambium, t =tunica

Table 1: Vegetative macroscopic features of *C. sieberiana*

Character	Fresh leaves
Habit	Tree
Leaf apex	Acute
Leaf base	Acute
Leaf margin	Entire
Leaf shape	Simple
Leaf color	Green
Leaf surface	Leathery, glossy
Leaf texture	Shiny
Leaf arrangement on petiole	Opposite
Leaf arrangement on stem	Opposite
Petiole	Present
Stipule	Deciduous
Midrib	Present, but more pronounced at the back
Stem color	Green

Table 2: Organoleptic properties of *C. sieberiana*

Parameter	Result
Texture	Coarse
Color	Army green
Taste	Bland taste
Odour	Characteristic odour

Table 3: Fluorescence analysis of powdered leaves of *C. sieberiana*

Powdered sample + reagents	Daylight	$UV_{254\ nm}$	$UV_{365\ nm}$
Sample + 50 % sulphuric acid	Green	Dirty green	Brown
Sample + 50 % hydrochloric acid	Dirty green	Dirty brown	Brown
Sample + 50 % nitric acid	Brown	Green	Dark brown
Sample + 50 % picric acid	Green	Light green	Brown
Sample + iodine	Green	Green	Brown
Sample + Acetic Anhydride	Brown	Green	Brown
Sample + 20 % KOH	Black	Black	Dark- green
Sample + chloroform	Green	Green	Brown
Sample + pet. Spirit	Green	Green	Brown
Sample + ferric chloride	Greenish- brown	Dark green	Black

Table 4: The extractive values of powdered leaves *C. sieberiana* using ethanol and distilled water.

Sample (s)	Weight of bottles (g)	Weight of bottle + extract (g)	Weight of extract (g)	Percentage yield
E1	71.00	71.05	0.05	5%
E2	65.26	65.29	0.03	3%
E3	60.50	60.56	0.06	6%
W1	66.97	67.02	0.05	5%
W2	62.05	62.10	0.05	5%
W3	64.83	64.10	0.07	7%

Where E1, E2 and E3 represents *C. sieberiana* powdered leaf sample in ethanol solution
W1, W2 and W3 represent *C. sieberiana* powdered leaf sample in distilled water.

Table 5: Percentage moisture content, total ash and acid insoluble ash of powdered leaves of *C. sieberiana*

S/N	% Moisture content	% Total ash	% Acid insoluble ash
1	8.95	3.52	0.52
2	8.97	3.50	0.50
3	8.92	3.50	0.52
4	8.95	3.50	0.54
5	8.95	3.48	0.50
6	8.95	3.50	0.50
7	8.88	3.52	0.52
8	8.94	3.51	0.50
Mean value	8.94±0.003	3.50±0.001	0.51±0.001

Data expressed as mean ± standard deviation.

Table 6: Chemo-microscopic analysis of powdered *C. sieberiana* leaves.

Chemo-microscopic tests	Observation
Test for lignin	Present
Test for oil	Present
Test for starch	Present
Calcium oxalate crystals test	Present
Test for tannin	Present

Table 7: Summary of the microscopic features of *C. sieberiana*

Parameters	Adaxial surface	Abaxial surface
Stomata	Present	Present
Trichomes	Present (numerous)	Present (scanty)
Epidermal cells	Present	Present
Palisade cells	Present	Present
Palisade ratio	2.0 – 3.25	

Table 8: Phytochemical screening of powdered leaves of *C. sieberiana*

Test	*Cassia sieberiana* leaf
1. Steroid (Salkowski's test)	
a) Leibermann- Burchard test	+
2. Terpenoids	-
3. Glycosides	++
4. Flavonoid Glycosides	
a) Ferric chloride	++
b) Lead acetate test	++
c) Shinoda test	++
5. Cardiac Glycoside	
a) Keller-killiani test	+
b) Kedde test	-
6. Saponin (foam test)	+++
a) Emulsion test	+++
7. Tannin	
a) Ferric chloride test	++
b) Lead acetate test	++
8. Anthocyanins	-
9. Coumarins	++
10. Alkaloids	

a) Wagner's reagent	++
b) Mayer's reagent	++
c) Dragendorff's reagent	++
11. Anthraquinone Glycoside (Borntrager's test)	++

Key: (+++) appreciably present, (++) moderately present, (+) slightly present, (-) absent.

Table 9: Antioxidant activity, total flavonoids and total phenolics contents of *C. sieberiana* leaves methanol extract and solvent fractions

Samples	IC_{50} (µg/mL)	TFC (mgQE/g)	TPC (mgGAE/g)
Crude methanol	293.99 ± 0.00	202.89 ± 0.09	6.81 ± 0.39
Hexane fraction	259.23 ± 20.89	202.59 ± 0.06	2.34 ± 0.40
DCM fraction	126.47 ± 4.99	202.80 ± 0.13	3.85 ± 0.34
Ethyl acetate fraction	71.42 ± 0.19	203.19 ± 0.03	25.10 ± 0.61
Rutin	20.6 ± 9.26		
Ascorbic acid	2.76 ± 0.001		

Data expressed as mean ± standard deviation.

DISCUSSION

Standardization is the process of guaranteeing that each dose of an ingredient has a specific amount, quality, and therapeutic effect [8]. With quality assurance techniques implemented in farming and industrial processes, standardization is accomplished by decreasing the intrinsic variance of the natural product's composition. The phytochemical and pharmacognostic analysis of several *Cassia sieberiana* components has already been the subject of a sizable amount of research in the last few years. This plant is a unique source of many different types of chemicals with different molecular structures. Phytochemical, physicochemical, microscopic, macroscopic (Organoleptic), and biological evaluations are some of the fundamental techniques frequently employed for assessing crude pharmaceuticals [2,6]. One useful method for identifying a species is via macroscopic examination.

Organoleptic evaluation is the study of pharmaceuticals using sense organs, including taste, size, texture, color, and odor [2,6]. Analysis of a sample's fluorescence is done by fluorescence analysis. A small quantity of the powdered sample was combined with several reagents, such as sulphuric acid, nitric acid, picric acid, iodine, etc., and the mixture was then studied in both daylight and UV light, with color changes noted at wavelengths of 254 nm and 365 mm. An essential pharmacognostic evaluation method for determining the presence or absence of contaminants is the physical-chemical analysis that was performed [30]. Moisture content, extractive value, ash value, and foreign organic matter are among the parameters that could be employed for physicochemical analysis. An accurate estimate of the weight of plant material can be obtained by measuring the moisture content. It is suggested that lower humidity levels improve product stability against deterioration [31]. Medicinal plants should have no more than 14% moisture content, according to British Herbal Pharmacopeia. Three methods are used to measure the amount of ash left over after medical materials are ignited: total ash, water-soluble ash, and acid-insoluble ash [6]. The powdered *Cassia sieberiana* leaves had a total ash value of 3.5% and an acid-insoluble ash value of 0.5%, respectively. The British Herbal Pharmacopeia states that 12% and 2.5%, respectively, are the maximum limits for total ash and acid-insoluble ash, respectively. The study's findings indicate that both the total ash and the acid-insoluble ash are within permissible limits. To obtain the medicine in its pure form, it is necessary to remove materials like sand, dust, and stone that are not from the plant of origin [32-34].

Extractive values are representative weights of the chemical components of crude drugs that can be extracted in various solvent environments [6,35,36]. It was noted that the plant yields the maximum percentage in water (7%) and the lowest percentage in ethanol (3%). The chemical evaluation includes qualitative and quantitative tests, chemical tests, chemical assays and instrumental analysis [6]. Microscopic evaluation allows for more detailed valuation of herbal drugs, in addition

to organoleptic evaluation and can be used to identify organized drugs based on their known histological characters [2]. A qualitative microscopic examination revealed that the plant possesses stomata, trichomes, polygonal epidermal cells, and palisade cells. However, the adaxial surface showed uniseriate, unicellular tector trichomes that are numerous while the abaxial surface showed sparsely distributed glandular trichomes. Also, the presence of anisocytic stoma on the adaxial surface is diagnostic for the identification of some Cassia species. The transverse section of *Cassia sieberiana* petiole indicated the presence of palisade cell, vascular bundle sheet, cortex, phloem, xylem and starch granule, while the longitudinal section features of *Cassia sieberiana* showed tector trichome, epidermal cell, leaf primordium and tunica.

Plant secondary metabolites include glycosides, flavonoids, saponins, phenols, tannins, coumarins, alkaloids, and anthraquinone glycosides were found via phytochemical screening, but sterols, terpenoids, and anthocyanins were not present. This indicates the biological activity that it would have. For instance, the antioxidant action it has may be ascribed to the presence of phenol and flavonoids, which are secondary metabolites. Natural antioxidants can be isolated from plants, which are a great supply of raw materials [37]. However, not one technique can reliably assess the antioxidant properties of plant extracts because of the phytochemical constituents' diversity and complexity. The Folin-Ciocalteu assay is widely employed in the measurement of total phenolic content. This procedure is based on the reduction of phosphotungstic and phosphomolybdic acid complexes to blue chromogens in alkaline conditions when phenolic chemicals are present [36]. The regression equation of the calibration curve of gallic acid ($y = 0.0052x+0.2864$; $R=0.9964$) is used to calculate the total phenolic contents. According to Rufino *et al.* [37], the content of total phenolics of crude extracts could be categorized into three classes: low (less than 10 mg GAE/g), medium (ranging from 10 mgGAE/g to 50 mgGAE/g) and high (more than 50 GAE/g). From the result gotten, it shows that ethyl acetate fraction has a moderate total phenolic contents of 25.10 ± 0.61 mg GAE/g while crude methanol, hexane and DCM fractions has low total phenolic contents with values of 6.81 ± 0.39, 2.34 ± 0.40 and 3.85 ± 0.34 mg GAE/g respectively. This may be due to the greater solubility of phenolic compounds in ethyl acetate than in the other fractions 35.

To estimate the total flavonoid concentration, the aluminum chloride test is frequently used [37, 38]. The foundation of this technique is the formation of acid-stable complexes between aluminum chloride and either the C-3 or C-5 hydroxyl group of flavones and flavonols, as well as the C-4 keto group. Apart from this, the ortho-dihydroxyl groups in the A or B ring of flavonoids also form acid-labile complexes with aluminum chloride. When these complexes combine with sodium nitrite in an alkaline environment, pink chromogens are the result [39]. The total flavonoid contents of crude extracts were determined from the regression equation of the calibration curve of Quercetin ($y=0.0013x+0.0958$; $R=0.9958$). From the result gotten, it was shown that the plant extract in all the solvent fractions (methanol, hexane, DCM, ethyl acetate) used had high total flavonoid content (202.89 ± 0.09, 202.59 ± 0.06, 202.80 ± 0.13, 203.19 ± 0.03 mgQE/g) with ethyl acetate fraction being relatively the highest. The DPPH assay is a commonly employed method in assessing the radical scavenging capacity of plant extracts [40, 41]. From this study, it was observed that ethyl acetate fraction had the lowest IC_{50} value (71.42 ± 0.19 µg/mL) of all the fractions used, compared to rutin with an IC_{50} value of 20.6 ± 9.26 µg/mL.

CONCLUSION

The macroscopic, microscopic and chemical tests carried on the powdered plant sample helps to determine the identity of the species as well as gives a clue of the biological effect it would exert. A qualitative microscopic examination revealed that *Cassia sieberiana* possesses stomata, trichomes, polygonal epidermal cells, and palisade cells. The presence of anisocytic stoma on the adaxial surface is diagnostic for the identification of *Cassia sieberiana*. The results of the study showed that the ethyl acetate fraction of *Cassia sieberiana* leaves methanol extract has a moderate total phenolic content and high total flavonoid content when compared to the other solvent extract used.

ACKNOWLEDGEMENTS: We appreciate all staff members of Department of Pharmacognosy, University of Ibadan, for providing the equipment used for this study.

CONFLICTS OF INTEREST: The article has no conflict of interest.

REFERENCES

[1] Bhusnure, O.G., Suryawanshi, S., Vijayendra, S., Gholve, S., Girm, P., Birajdar, M., (2019). Standardization and Quality Evaluation of Herbal Drugs. Journal of Drug Delivery and Therapeutics 9(3-s): 1058-1063.

[2] Barnes, J., Anderson, L.A., Phillipson J.D., (2007). Herbal medicine. 3rd Edition, Press, London: Page 1-23.

[3] Tanaka, N and Kashiwada Yoshiki., (2021). Phytochemical studies on traditional herbal medicines based on the ethnopharmacological information obtained by field studies. Journal of Natural Medicines. 75(4): 762-783.

[4] Sachan, A., Garima, V., Roopak, K., (2016). Need of standardization of herbal medicines in Modern era. International Journal of Phytomedicine 8(3): 300.

[5] Bijauliya, R.K., Shashi, A., Dilip, K.C., Mayank, K., (2017). A comprehensive review on standardization of herbal drugs. International Journal of Pharmaceutical Sciences and Research volume 8(9): 3663-3677.

[6] Sekham, BS (2011). An overview of advances in the standardization of herbal drugs. Journal of Pharmaceutical Education Resource, 2(2): 55-64.

[7] Kunle, Oluyemisi Folashade, Egharevba, Henry Omoregie and Ahmad, Peter Ochogu (2012) Standardisation of herbal medicine- A review. International Journal of Biodiversity and Conservation Vol 4(3):101-112.

[8] Pharm-Huy, LA., Hua He., Pharm-Huy, C., (2008). Free Radicals, Antioxidants in Disease and Health. International Journal of Biomedical Science 4(2): 89-96.

[9] Liu, Z.-Q. (2009). Bridging free radical chemistry with drug discovery: a promising way for finding novel drugs efficiently. European Journal of Medicinal Chemistry 189: 112020.

[10] Keservani, RK., Sharma, AK and Kesharwani, RK., (2016). Medicinal Effect of Nutraceutical Fruits for the Cognition and Brain Health.

[11] Mukhtar, H., Ahmad, N., (2000). Tea polyphenols: prevention of cancer and optimizing health. National Library of Medicine 71(6): 1698-1703.

[12] Zargoosh, Z., Ghavam, M., Tavili, A., (2019). Effects of ecological factors on the antioxidant potential and total phenol content of *Scrophularia striata* Boiss. *Scientific Reports* 9: 16021.

[13] Aminjan, H.H., Abtahi, S.R., Hazrati E., Chamanara, M., Jalili, M., Paknejad., (2019). Targeting of oxidative stress and inflammation through ROS/ NF-kappaB pathway in phosphine-induced hepatotoxicity mitigation. *Life Science.* 232: 116607.

[14] Reuter, S., Gupta, S.C., Chaturvedi, M.M., and Aggarwal, B.B. (2010). Oxidative stress, inflammation and cancer: how are they linked? *Free Radical Biological Medicine* 49: 1603-1616.

[15] Von Maydell, Hans-Jurgen (1990). Trees and Shrubs of the Sahel: Their Characteristics and Uses Verlag Josef Margraf, Scientific Books.

[16] Okpoko, C., Ezenyi, I., Adzu, B., Salawu, O., (2020). Evaluation of two medicinal plants used for arthritis in northern Nigeria with focus on *Terminalia avicennioides* Guill. & Perr. and its mechanism of action. Scientific African. Volume 8.

[17] Dougnon, V., Assogba, P., Koudouvo, N., Ohouko, O., (2020). An ethnopharmacological survey about the Togolese plants used in the treatment of infectious diseases: a way to explore new substances. Annals of Ayurvedic Medicine 9(2): 85.

[18] Leteane, M., (2012). “Old plants newly discovered: *Cassia sieberiana* D.C. and Cassia abbreviate Olic. Oliv. Root extracts inhibit in vitro HIV-1c replication in peripheral blood mononuclear cells (PBMCs) by different modes of action. J. Ethnopharmacol” 141(1): 48-56.

[19] Nuhu Abdulrazak, Umar Imam, NataaLa Shehu, Aminu Farida, (2015). Antiplasmodic activity of ethanolic extract of root and stem back of *Cassia sieberiana* DC on mice. Journal of Intercultural Ethnopharmacology. 4(2): 1.

[20] Kokate, CK., Purohit, AP., Gokhale, SB., (2002). Textbook of Pharmacognosy. 18th edition. Pune: Nirali Prakashan.

[21] Ogunlakin, AD, Ojo, OA, Gyebi, GA, Adebodun, GO, Elbasyouni, A, Adebodun, SA, Ogunlakin, B, Olanrewaju, AA, Sonibare, MA., (2024). Effect of Triclisia subcordata Oliv. (Menispermaceae) leaves on hormonal imbalance and genes expression in the ovaries of letrozole-induced polycystic rats via in vivo and computational approaches. Journal of Molecular Structure. 1318 (2024): 139275

[22] Lala, PK., (1981). Practical Pharmacognosy. 1st edition. Calcutta: Lina.

[23] Chase, CR., Pratt, R., (1949). Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. Journal of America Pharmacology Association. 38(6): 324-31.

[24] Harbone, JB., (2005). Phytochemical methods-A guide to modern techniques of plant analysis. Berlin: Springer Verlag; 2005.

[25] Lawal, AM., Lawan, M.M., Apampa, S.A., (2019). Phytochemical Analysis and Thin Layer Chromatography Profiling of Crude Extracts from Guiera Senegalensis (Leaves). Biotechnology biomedical science. 3(3): 7-12.

[26] Solanki S., Modi CM., Patel HB., Patel UD., (2019). Phytochemical screening and thin-layer chromatography of six medicinal plants from the surroundings of Junagadh, Gujarat, India. *J Pharmacogn Phytochem*. 8(4):3122–3126.

[27] Wilde TJJD., Ben IO., Woode E., Abotsi WKM., (2013). Preliminary phytochemical screening and in vitro antioxidant properties of Trichilia monadelpha (Thonn.) JJ De Wilde (Meliaceae). *J Med Biomed Sci*. 2:6–15.

[28] Adeniran, A.A., Sonibare, M.A., (2013). In vitro potential antihelminthic activity of bulbis of Dioscorea bulbifera L. on earthworms and liverflukes. Journal Pharmacognosy Phytother 5(12):196 -203.

[29] Parnika, C., Rakesh K., (2020). Standardization and Evaluation of Herbal drugs is need of the hour in present era. Plant Archives 20(2): 7883-7889.

[30] Balekundri, A. and Mannur, V., (2020). Quality control of the traditional herbs and herbal products: a review. Future Journal of Pharmaceutical Sciences 6: 67.

[31] Majid, M., Khan, JN., Shah, QMA., Masoodi, KZ., Afroza, B., Parvaze, S., (2021). Evaluation of hydroponic systems for the cultivation of Lettuce (Lactuca sativa L., var. Longifolia) and comparison with protected soil-based cultivation. Agricultural water Management. Volume 245.

[32] Quality control methods for herbal materials. Geneva: World Health Organization; 2011.

[33] Kshirsagar, RB., Sharma, D., Sawate, AR., (2017). Studies on physico chemical characteristics of Gymnema sylvestre (Leaf, powder and extract). Journal of Pharmacognosy and Phytochemistry 6(5): 250-255.

[34] Patel, V., Prakash, KD., Shivaji, GS., Rohidas, KP., Bhalke, R., (2017). Current quality control methods for standardization of herbal drugs. *International Journal of Pharmaceutics and Drug Analysis* 5(3): 82-95.

[35] Patel, J., Khogta, S., Kalyani, B., Vaishali, L., (2020). Herbal nano-formulations for topical delivery. *Journal of Herbal Medicine.* Volume 20.

[36] Wang Z, Mei X, Chen X, Rao S, Ju T, Li J, Yang Z. Extraction and recovery of bioactive soluble phenolic compounds from brocade orange (Citrus sinensis) peels: Effect of different extraction methods thereon. LWT. 2023 Jan 1;173: 114337.

[37] Mehran, M.J., Zendehbad, S.H., and Malla, S., (2014). Free radical scavenging and antioxidant potential activity of cassava plants. Asian Journal of Pharmaceutical and Clinical Research 7(1):66-70.

[38] Corpuz, M.J.A.T., Osi, M.O., and Santiago, L.A. (2013). Free radical scavenging activity of Sargassum aliquosum J.G. Agardh, International Food Research Journal, 20(1):291-297.

[39] Rajanandh, M.G., and Kavitha, J., (2010). Quantitative estimation of B-sitosterol, total phenolic and flavonoid compounds in the leaves of Moringa oleifera. International Journal of Pharmatech Research, 2(2):1409-1414.

[40] Ndala, AR, Mayo, M., and Staden, J.V., (2010), Natural antioxidants: fascinating or

mythical biomolecules? Molecules, 15(10):6905-6930.

[41] Murali, A., Ashok, P., and Madhavan, V. (2011). In vitro antioxidant activity and bptic studies on the roots and rhizomes of Smilax zeylanica L. (Smilacaceae), International Journal of Pharmacy and Pharmaceutical Sciences, M1):192-195.