Pharmacognostic studies on stem bark of *Canarium zeylanicum* (Retz.)

Pathirana M.P.K.N.[1,2*], Kumarapeli K.A.M.S.[2], Dayananda T.G[1], Wijayasiriwardena T.D.C.M.K.[2]

Abstract

Canarium zeylanicum (Retz.) of the family Burseraceae, is an endemic medicinal plant of Sri Lanka which is used for medicinal purposes and as a fumigant to repel mosquitoes for ages. Though, it is endemic, a large number of plants are available throughout the wet zone which can be sustainably utilized for the wellbeing of the public. The present study aims to establish data on macroscopical, microscopical, and certain phytochemical and physicochemical characteristics of stem bark and stem bark powder. Three representative samples were collected from three geographical locations of Sri Lanka and then macroscopical, and microscopical characteristics were investigated. Preliminary phytochemical screening was done on the bark powder after the sequential extractions on hexane, dichloromethane (DCM), and methanol, followed by the development of Thin Layer Chromatography (TLC) fingerprints for the above extracts. Total terpenoid content was estimated in the hexane fraction. Physico-chemical characteristics; total ash, acid-insoluble ash, and moisture contents were analysed. The essential oil content of the bark was determined using the Clevenger apparatus. Microscopically, an abundance of groups of pitted lumen stone cells, crystal fibres, and the presence of prismatic crystals of Calcium oxalate were characteristic anatomical features in the cortical region. Alkaloids, saponins and tannins were observed in methanol fraction and terpenoids in hexane and DCM fractions. Total terpenoid content, total ash and acid insoluble ash contents were 391.84±3.98 mg/g, 9.72±0.07%w/w and 5.00±0.05%w/w respectively. Moisture content was 9.96±0.03%v/w on a wet basis and the essential oil content was 0.26±0.01%v/w on a dry weight basis. Data generated by the present study may be useful to determine the quality and purity of the stem bark of Canarium zeylanicum (Retz.).

Keywords: *Canarium zeylanicum* (Retz.), TLC, Burseraceae, Pharmacognosy, Terpenoids

Introduction

The plant *Canarium zeylanicum* (Retz.), a species of flowering plant in the family Burseraceae, is endemic to Sri Lanka. In Sinhala this plant is known as "*Dik Kekuna*" and in Tamil, it is known as *Skillil* (*Pakillipal*)[1].

Canarium zeylanicum (Retz.) is a large branched tree that grows up to 25–30m in height. It has imparipinnate compound leaves, arranged alternatively on the stem branches 1 and green colour flowers, arranged in terminal panicles on short, stout pedicels. The peel of the nut is thick and copper in colour. In the centre of the fruit, there is 1-2 seed covered by white flesh. The seed contains a high-fat white kernel. The bark is about 4mm thick, hard, and pale brown in colour. Gum is naturally occurring in the plant stem and when the bark is injured, an abundance of a beautifully clear, fragrant, balsamic gum resin exudes from it and this is used for fumigation in houses against mosquitoes for ages[2].

Medicinally, the bark of the tree is astringent and antiseptic. A decoction of it is used as a gargle for bleeding and spongy gums. An ointment prepared

[*1]*Department of Botany, Faculty of Science, University of Ruhuna, Matara, Sri Lanka.*
[2] *Herbal Technology Section, Industrial Technology Institute, Malabe, Sri Lanka.*

**Correspondence: Pathirana M.P.K.N., Department of Botany, Faculty of Science, University of Ruhuna, Matara, Sri Lanka. Email: kalpani1515@gmail.com*

by boiling bark with Coconut oil is used as a dressing for chronic ulcers. Internally, it is used as an aromatic stomachic and astringent and is commonly used against diabetes[2].

Although the stem bark is used for a variety of therapeutic purposes in traditional medicine and as a fumigant against mosquitoes for ages, there is no detailed Pharmacognostic data reported on the bark of *Canarium zeylanicum* (Retz.) plant. Therefore, the present study aims to establish Pharmacognostic data on the stem bark of *Canarium zeylanicum* (Retz.), for its authentication and to develop value-added standardized products with commercial importance, based on the stem bark.

Materials and Methods

Collection of plant materials

Canarium zeylanicum (Retz.) stem barks were collected from matured plants at Bulathsinghala of Kaluthara district, Deiyandara of Matara district, and Dambadeniya of Kurunegala district in Sri Lanka. The authenticity of plant material was accessed by comparing it with local floras available in textbooks at the Pharmacognosy laboratory of the Industrial Technology Institute, Malabe, Sri Lanka.

Macroscopical and microscopical studies

Preparation of samples

For macroscopical and microscopical studies thoroughly washed stem barks were cut into desired sizes (7cm-9cm) and preserved in formaldehyde acetic acid and alcohol solution (FAA). For powder microscopical studies dried stem bark samples were grinded, sieved and 120 mesh powder was obtained and stored in air-tight containers until used for further analysis[3].

Macroscopical analysis

Macroscopic character evaluation of the bark and powder samples were carried out by examining its colour, odour, texture and appearance.

Microscopical analysis

Method of slide preparation

Freehand sections of stem bark were taken using a sharp blade and were mounted with Chloral hydrate solution to observe various anatomical features. Specimen slides, so prepared were observed under mid-power 10x followed by a high-power 40x of "Labomed Sigma" compound microscope. For powder, microscopical identification 120 mesh powder was mounted with Chloral hydrate and observed as earlier[4,5].

Determination of phytochemical characteristics extraction of the samples

Dried bark powder (50g) was sequentially extracted with hexane, dichloromethane, and methanol as solvents by hot Soxhlet extraction method[6].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out for each extract of sequential extractions using standard protocols to identify constituents [7,8,9,10,11].

Development of Thin Layer Chromatography profiles

The sequential extracts obtained from Soxhlet extractions were spotted on pre-coated silica gel 60G F_{254} aluminum plates. Different solvent systems were used for methanol, DCM, and hexane extracts to obtain a clear separation of compounds as Table 1.

Table 1: Development of Thin Layer Chromatography profiles with different solvent systems

Type of extract	Solvent system ratio			
	Methanol(v/v)	DCM (v/v)	Hexane (v/v)	Formic acid (v/v)
Methanol extract	4	3	1	0.5
DCM extract	5	5	1.5	0.5
Hexane extract	5	5	2	0.5

Then TLC profiles were developed and the retention factors (Rf) were calculated at wavelength 254nm, 366 nm, and after spraying vanillin sulphuric[12,4,5].

Determination of Total Terpenoid Content (TTC)

Initially, 10 g of *Canarium zeylanicum* (Retz.) bark powder was subjected to Soxhlet extraction with hexane and bark powder hexane extract was obtained. Then TTC was determined using 300mg of bark powder hexane extract according to standard protocols using Linalool as standard [13,14,15].

Determination of physico-chemical characteristics

Total ash, acid insoluble ash, and moisture content of the powder samples were determined according to standard protocols [16,17,18].

Determination of essential oil content

Initially, 100.00 g of bark powder was subjected to hydro distillation using Clevenger-type apparatus, and essential oil content was determined [19,20].

Results

Macroscopical characters

Canarium zeylanicum (Retz.) stem bark samples were 6 to 7mm in thickness, externally rough, fracture was fibrous and light brown in colour with a characteristic odour. The transverse section of the stem bark was pale brown in colour and the bark powder was light brown with a characteristic aromatic odour.

Microscopical characters

In the cork region hexagonal thin-walled parenchyma cells most filled with brown colouring matter and a few groups of stone cells, thick-walled fibres were also observed.

In the cortical region; an abundant group of stone cells with pitted lumen and large groups of thick-walled fibres were observed. Some fibre cells were associated with prismatic crystals of Calcium oxalate as crystal fibres and it was identified as a characteristic feature of the stem bark. Long fibres and short fibres were abundant and amongst them some were wide-lumen, short fibres. The abundance of isolated prismatic crystals of Calcium oxalate was observed among stone cells and thick-walled fibres. Isolated and compound starch grains were also found in the cortical region and vessels with spiral arrangement and biseriate to multiseriate medullary rays were observed in the cross section of stem bark cortical region of *Canarium zeylanicum* (Retz.) (Plate 1).

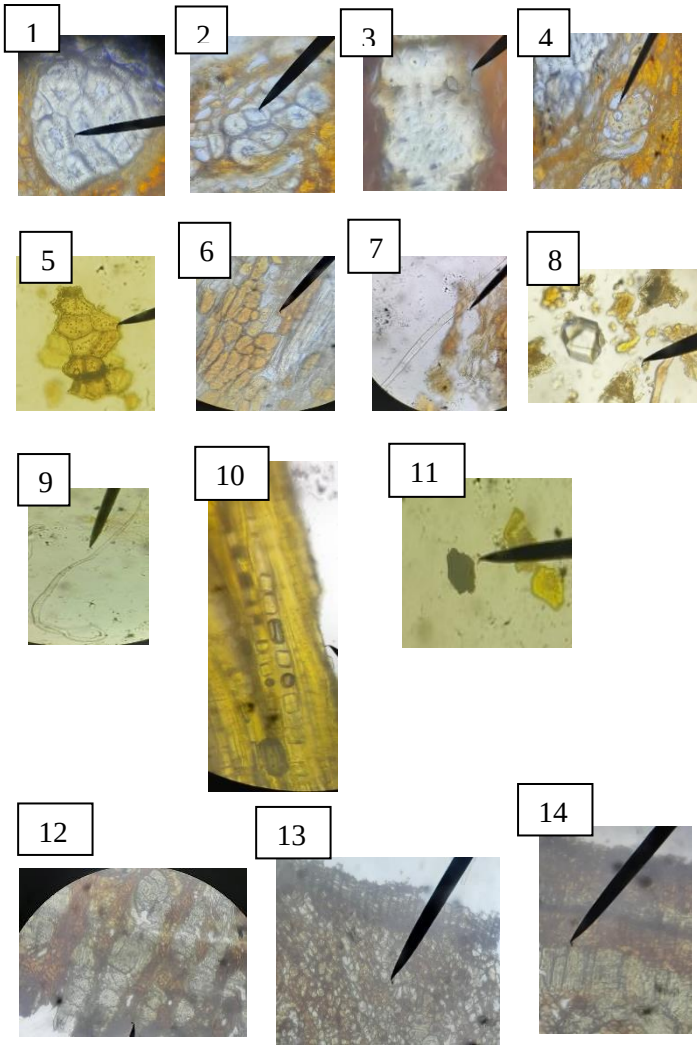

Plate 1: Photographic images of microscopical studies (Labomed Sigma" USA compound microscope)

1) Stone cells 2) Stone cells associated with Calcium oxalate prismatic crystal 3) Thick walled fibres in sectional view 4) Thick walled fibres in sectional view associated with a prismatic crystal 5) Pitted walled fibres in sectional view 6) Essential oil filled cells 7) Wide lumen short fibres 8) Prismatic crystal 9) Long fibres 10) Calcium oxalate prismatic crystals associated fibres 11) Starch grain
12) Transverse section of the cortical region
13) Transverse section of the cork region
14) Transverse section of the bark

Preliminary phytochemical study

Preliminary phytochemical studies revealed the presence of alkaloids, tannins, and saponins in methanol extract and terpenoids in hexane and dichloromethane extract (Table 1).

Table 1: Preliminary phytochemical screening of stem bark powder extract of *Canarium zeylanicum* (Retz.)

Phyto chemicals	Hexane extract	Methanol extract	Dichloromethane extract
Alkaloids	-	+	-
Flavonoids	-	-	-
Tannins	-	+	-
Saponins	-	+	--
Terpenoids	+	-	++
Steroids	-	-	-

++ (Present highly), + (Present), - (Absent)

Thin Layer Chromatography (TLC) profiles of Canarium zeylanicum (Retz.) stem bark powder extracts

Thin Layer Chromatography (TLC) profiles of *Canarium zeylanicum* (Retz.) stem bark powder extracts with DCM extract, Methanol extract and Hexane extract are shown in plate 2(A), 2(B) and 2(C).

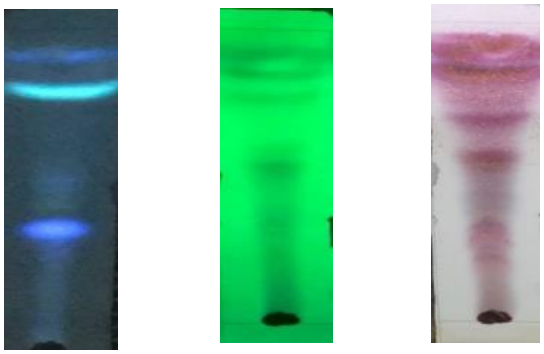

Plate 2(A): TLC fingerprint profile of DCM extract

A1) at 365nm A2) at 254nm A3) After derivatisation using Vanillin sulphuric

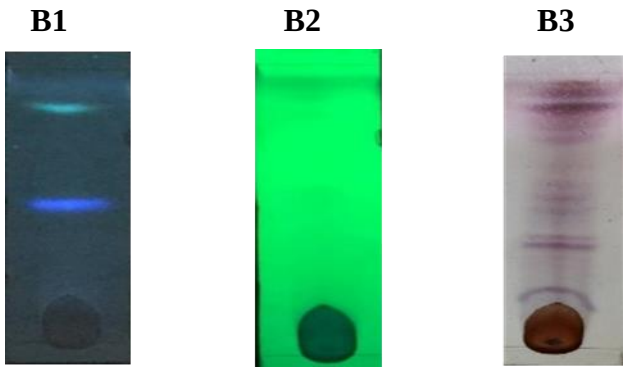

Plate 2(B): TLC fingerprint profile of Methanol extract

B1) at 365nm B2) at 254nm B3) After derivatisation using Vanillin sulphuric

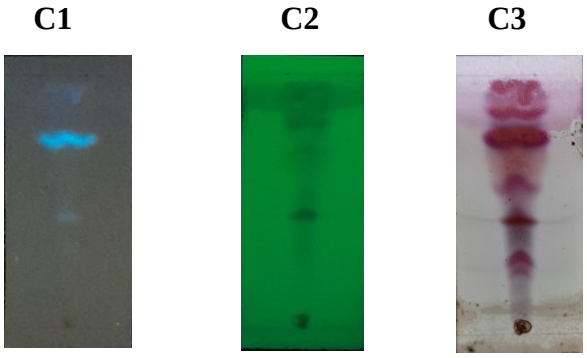

Plate 2(C): TLC fingerprint profile of Hexane extract

C1) at 365nm C2) at 254nm C3) After derivatisation using Vanillin sulphuric

TLC details of Canarium zeylanicum (Retz.) stem bark powder extracts

TLC studies established chromatographic patterns for *Canarium zeylanicum* (Retz.) stem bark powder dichloromethane, methanol, and hexane extracts. After derivatisation using vanillin sulphuric, dichloromethane extract with solvent system DCM, hexane, ethyl acetate, formic acid has shown fifteen coloured spots, methanol extract with solvent system DCM, cyclohexane, ethyl acetate, formic acid has shown twelve coloured spots and hexane extract with solvent system DCM, hexane, ethyl acetate, formic acid has shown twelve coloured spots (Table 2,3 and 4).

Table 2: TLC details of *Canarium zeylanicum* (Retz.) stem bark powder extracts (solvent system DCM)

Dichloromethane (DCM) extract

Rf values at day light	Rf values at UV light		Rf values after derivatisation
	254nm	365nm	
0.92	0.24	0.38	0.19-Brown
	0.29	0.45	0.24-Light
	0.32	0.54	purple
	0.38	0.81	0.26-Pink
	0.50	0.92	0.29-Orange
	0.56		0.35-Yellow
	0.75		0.48-Pink
	0.81		0.49-Blue
	0.92		0.54-Brown
			0.66-Purple
			0.76-Brown
			0.78-Blue
			0.80-Pink
			0.86-Pink
			0.90-Brown
			0.92-Pink

Table 3: TLC details of *Canarium zeylanicum* (Retz.) stem bark powder extracts (solvent system DCM)

Methanolic extract

Rf values at daylight	Rf values at UV light		Rf values after derivatisation
	254nm	365nm	
	0.81	0.50	0.21-Purple
	0.86	0.78	0.75-Purple
	0.92	0.91	0.38-Purple
			0.80-Purple
			0.40-Purple
			0.86-Violet
			0.5-Blue
			0.92-Violet
			0.55-Purple
			0.94-Violet
			0.60-Purple
			0.66-Light brown

Table 4: TLC details of *Canarium zeylanicum* (Retz.) stem bark powder extracts (solvent system DCM)

Hexane extract

Rf values at day light	Rf values at UV light		Rf values after derivatisation
	254nm	365nm	
	0.32	0.32	0.25 Purple
	0.43	0.43	0.28-Pink
	0.73	0.52	0.32-Violet
	0.92	0.68	0.45-Brown
	0.95	0.80	0.46-Blue
		0.83	0.58-Pink
		0.91	0.65-Pink
			0.66-Orange
			0.78-Pink
			0.85-Pink
			0.92-Pink
			0.98-Pink

Physiochemical characteristics, essential oil content and total terpenoid content of stem bark powder of *Canarium zeylanicum* (Retz.)

Physio-chemical characteristic values, essential oil content and total terpenoid content of *Canarium zeylanicum* (Retz.) bark powder is shown in Table 5.

Table 5: Physicochemical values, essential oil content and total terpenoid content of *Canarium zeylanicum* (Retz.) stem bark powder

Parameter	value
Total ash content%(w/w)	9.72±0.07%
Acid insoluble ash content%(w/w)	5.00±0. 0.05%
Moisture content%(w/w)	9.96±0.03%
Essential oil content%(v/w)	0.26±0.01%
Total terpenoid content (TTC)	391.84±3.98mg Linalool equivalent /g

Results are represented as mean ±SE(n=3)

Discussion

The macroscopical and microscopical analysis is one of the cheapest method, that can be used as an identifying parameter to substantiate and authenticate a drug [21]. Therefore, observed macroscopical and microscopical characteristics of stem bark and bark powder would be a useful tool for plant identification.

Phytochemical analysis reveals useful findings about the chemical nature of the stem bark powder. Tannins, saponins, alkaloids, which were found in stem bark extracts are known to have beneficial medicinal properties while terpenoids are known to have anti-inflammatory, antiviral, and insect-repellent activity[15,22,23]. For phytochemical analysis plant extracts were obtained using the Soxhlet extraction method because it makes the extraction process much more efficient with a high extraction yield that requires less time and solvent consumption than maceration or percolation [24]. First, bark powder was extracted with nonpolar hexane as the solvent, then with dichloromethane as the solvent and later with the methanol according to the polarity increasing order resulting in sequential extraction. Sequential extraction ensures the extraction of all active components according to their polarity order [6]. Thin Layer Chromatography (TLC) is an affinity-based technique used to separate the constituents of a mixture [25]. Results of TLC fingerprint profiling can be used as a quality standard for purity determination of Canarium zeylanicum (Retz.) bark powder.

Physicochemical parameter determination is an important criterion to judge the identity or purity of crude drugs. Ash contents reveal how much minerals are physiologically contained in the medicinal plants and how many foreign materials have been mixed in during the course of processing [16]. According to the obtained data total ash content was within the average range compared to other plants. But acid insoluble ash content has much higher values and can be assumed that, it is due to contamination of bark powder with silica crystals. The moisture content was determined using Dean and Stark method because bark powder contains volatile oils which makes the conventional oven drying method not applicable. As obtained results moisture content of the bark was within the acceptable range of 8 - 12% for minimum bacteria, and fungal growth [26].

The essential oil yield of plants is often below 1%, rarely reaching 10% or even more in some dry plant parts. The plants which contain at least 0.1%-0.2% volatile oil content with a perceptible odour is considered as economically viable aromatic plants. The essential oil content of Canarium zeylanicum (Retz.) bark powder is within the range and can be used to develop value-added aromatic products.

Conclusion

Through this study, identification data of *Canarium zeylanicum* (Retz.) stem bark and bark powder were established based on macroscopical and microscopical characteristics. Quality standards for *Canarium zeylanicum* (Retz.) stem bark powder has been established with respect to certain physicochemical and phytochemical characteristics. The data revealed in this study can be used as a reference to determine quality and the purity of *Canarium zeylanicum* (Retz.) stem bark in future studies and for the development of herbal-based quality products.

Acknowledgement

Acknowledged to Treasury Grant 19/175 of Industrial Technology Institute

References

1. Tisseverasinghe, A.E.K. (1971), *A manual of timber utilization for Ceylon*, The Ceylon Forest department, 1st ed, Sri Lanka, 78.
2. Jayaweera, D. M. A. (1981), *Medicinal plants (indigenous and exotic) used in Ceylon*, The National Science council of Sri Lanka, Sri Lanka, 206-207.
3. Alam, F., & Saqib, N.Q. (2015). Pharmacognostic standardization and preliminary phytochemical studies of *Gaultheria trichophylla*. *Pharmaceutical Biology*. 53(12): 1711-1718.

4. Saraswathy, A., Meena, A. K., Shakila, R., Sunil Kumar, K. N., & Ariyanathan, S. (2010). Pharmacognostic studies on *Alangium salvifolium* (Linn.f.) Wang. root bark. *Pharmacognosy Journal*. *2*(11): 374–380.
5. Singh, D., Aeri, V., & Ananthanarayana, D.B. (2018). Development of Standard Operating Protocol for Slide Preparation of Powdered Bark Samples with Varying Grinding Techniques. *Pharmacognosy Journal*. 10(2): 265-271.
6. Abubakar, A.R. & Haque, M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of Pharmacy and Bio allied Sciences.*12(1): 1-10.
7. Bulugahapitiya, V.P. (2008), *Plant based Natural products Extraction, Isolation and Phytochemical Screening*, Bulugahapitiya V. P.,1 st ed., Sri Lanka, 83-89.
8. Muthuswamy, R., & Senthamarai, R. (2014). Pharmacognostical studies on stem bark of *Canarium strictum* Roxb. *Pharmacognosy Journal*. 6: 12-18.
9. Roopashree, T. S., Dang, R., Rani, R. H. S., & Narendra, C. (2008). Antibacterial activity of antipsoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. *International Journal of Applied Research in Natural Products*. 1: 20–28.
10. Sofowora, A. (2008), *Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa*, Spectrum Books Ltd, 5 th ed., Nigeria, 150-156.
11. Trease, G. E., & Evans, W. C. (2009), Phenols and phenolic glycosides, *Textbook of Pharmacognosy*, Tindall and Co Publishers, 16 th ed., London, 343–383.
12. Ahmed, H. M., Ramadhani, A. M., Erwa, I. Y., Ishag, O. A., & Saeed, M. B. (2020). Phytochemical screening, chemical composition and antimicrobial activity of *Cinnamon verum* Bark. *International Research Journal of Pure and Applied Chemistry*. 21(11):36-43.
13. Ghorai, N., Chakraborty, S., Gucchait, S., Saha, S., & Biswas. (2012). Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. *Protocol Exchange*. 1-5.
14. Harman-Ware, A. E., Sykes, R., Peter, G.F., & Davis, M. (2016). Determination of Terpenoid Content in Pine by Organic Solvent Extraction and Fast-GC Analysis. *Frontiers in Energy Research*.4:2
15. Indumathi, C., Durgadevi, G., Nithyavani, S., & Gayathri, P. K. (2014). Estimation of terpenoid content and its antimicrobial property in *Enicostemma litorrale. International Journal of Chem Tech Research*. 6(9): 4264-4267.
16. Gruber, L., Seidl, L., Zanetti, M., & Schnabel, T. (2021). Calorific Value and Ash Content of Extracted Birch Bark. *Forests*. 12: 1480.
17. Pande, A. (1974), *Handbook of Moisture Determination and Control*, Marcel Dekker, 3rd ed., New York, 1.
18. Park, Y. W., & Bell, L. N. (2004). Analysis of moisture contents of foods. *Handbook of Food Analysis: Physical characterization and nutrient analysis*,Marcel Dekker, 2nd ed., New York, 55-82.
19. Shakeri, A., Khakdan, F., Soheili, V., Sahebkar, A., Rassam, G., & Asili, J. (2014). Chemical composition, antibacterial activity, and cytotoxicity of essential oil from *Nepeta ucrainica* L. ssp. *Industrial Crops and Products*. 58: 315–321.

20. Unlu, M., Ergene, E., Unlu, G. V., Zeytinoglu, H. S., & Vural, N. (2010). Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from *Cinnamomum zeylanicum* Blume (Lauraceae). *Food Chem Toxicol.*48(11): 3274–3280.
21. Serrano, R., Silva, G., & Silva, O. (2010). Application of Light and Scanning Electron Microscopy in the Identification of Herbal Medicines. In A. Méndez-Vilas & J. Diaz (Eds.), *Microscopy: Science, Technology, Applications and Education*, Formatex, 4th ed., Badajoz, 182–190.
22. Okwu, D.E., & Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology*. 5(4): 357-361.
23. Sodipo, O.A., Akiniyi, J.A, & Ogunbamosu, J.U. (2000). Studies on certain on characteristics of extracts of bark of Pausinystalia johimbe and Pausinystalia macroceras (K Schum) Pierre ex Beille. *Global Journal of Pure and Applied Sciences*. *6*(1): 83-87.
24. Zhang, Q.W., Lin, L.G., & Ye, W.C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine (United Kingdom)*. BioMed Central Ltd. 13:20.
25. Bhusnure, O.G., Suryawanshi, S., Vijayendra Swamy, S.M., Gholve, S.B., Girm, P.S., & Birajdar, M.J. (2019). Standardization and Quality Evaluation of Herbal Drugs. *Journal of Drug Delivery and Therapeutics*. 9(3-s): 1058-1063.
26. Seth, S., Agrawal, Y.C., Ghosh, P.K., & Jayas, D.S. (2010). Effect of moisture content on the quality of soybean oil and meal extracted by isopropyl alcohol and hexane. *Food and Bioprocess Technology*. *3*(1): 121-127.