World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 3.454
Year - 2024
Volume: 3; Issue: 4
Page: 113-121

DIFFERENTIATION OF TWO ALLIED PINK CASSIA SPECIES BASED ON PHYSICOCHEMICAL AND CHEMOTAXONOMIC STUDIES

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Article Received: 05 June 2024 || Article Revised: 26 June 2024 || Article Accepted: 19 July 2024

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ABSTRACT

Cassia javanica Linn. and *Cassia nodosa* Buch.-Ham. ex Roxb. are popular garden plants from family Fabaceae (Leguminosae). Apart from ornamental use, these plants yield commercially important wood and seed gum. Both plants are less explored for therapeutic potential and recently leaves of them are proved to be antidiabetic. Morphologically *C. javanica* and *C. nodosa* are very much similar. In addition to this, being ornamental plants, the taxonomic data of these plants is insufficient for correct identification. In order to find out quick simple identification character/s, elaborate study involving various pharmacognostic characters was done. Firstly, Macroscopical and microscopical studies were done using leaves and leaflets. In second step, dried leaflet powders were subjected to physicochemical and preliminary phytochemical analysis. Further crude leaf extracts were investigated with thin layer chromatography (TLC) and quantitative phytochemical evaluation. It was found that, the said plants resemble in many external and internal characteristics but the distinct results were obtained in physicochemical, quantitative phytochemical and TLC analyses. This study indicated that a simple TLC analyses of anthraquinone glycosides and saponins along with physicochemical constants and relatively different phytochemical contents can be used as reliable identification keys.

KEYWORDS: Anthrquinone glycosides, *Cassia javanica*, *Cassia nodosa*, Saponins, TLC.

INTRODUCTION

Genus *Cassia* comprises a large group (about 500 species) of flowering plants. In folk medicines, they are mainly used for laxative and purgative action.[1] *Cassia javanica* Linn. and *Cassia nodosa* Buch.-Ham. ex Roxb. are well known for their graceful appearance with beautiful racemes of delicate pink flowers. Both these Pink Cassias are medium sized

trees belonging to family – Fabaceae (Leguminosae), subfamily – Caesalpinae which form centre of attraction in gardens of major cities of India.

***Cassia javanica* Linn.** is a native of Java, Malaysia, Indonesia and Sumatra. It is a small to medium sized deciduous tree, up to 6 to 10 m tall; straight short trunk; fairly smooth dark brown bark; supporting a spreading crown. The wood is used in construction of houses, furniture and cabinet work. The seeds are used for production of gum. It is an industrially useful natural product utilized in tablet formulations.[2-5] The phytochemical screening of the crude methanolic stem extracts of *Cassia javanica* plant showed the presence of different classes of organic compounds like alkaloids, tannins, flavonoids, saponins, phlobatanins, steroids, anthraquinone and cardiac glycoside.[6] The plant also has a few medicinal properties. The pods are used as a substitute for *C. fistula* in French Guiana. They are purgative and show haemoagglutinating action.[3,7-11] The therapeutic uses for leaves of *C. javanica* are not reported in previous literature. A unique compound ent–epiafzelechin–(4α→8)–epiafzelechin extracted from the fresh leaves of *C. javanica* inhibits *Herpes simplex* virus type 2, thus pharmacologically proved to be antiviral in action.[12] Preclinically, water extract of leaves exhibited significant hypoglycaemic action on Wistar Albino Rats.[13]

***Cassia nodosa* Buch.-Ham. ex Roxb.** is a small to medium sized tall tree, 10 m to 20 m in height, deciduous to evergreen, taller than *C. javanica* Linn.; knotted trunk, greyish or yellowish brown bark with narrow, deep horizontal clefts; canopy globose to sub conical with numerous drooping feathery leafed branchlets. Leaves of *C. nodosa* contain anthraquinones such as rhein, aloe-emodin, chrysophanic acid in free or combined form, rhein glucoside, chrysophanol, physcion, kaempferol, tetramethyl ether of kaempferol, kaempferol 3-O-rhamnoside, flavones, quercetin-3-O-arabinoside and 5,4-dihydroxy-7-methyl 3-benzyl chromone (1). A very few phytoconstituents are also reported from flowers.[9,11,14-16]

The wood is used for cabinet work, furniture, beams, posts, handles of axes, knives and general construction. The roots are used as laundry soap. Leaves are purgative, though sometimes eaten with rice in South-East Asia, used for poulticing boils. The boiled leaves are used as refrigerant. As leaves contain alkaloids, are fatal to livestock. The yellowish brown smooth bark contains a saponin, useful in itching skin. The seeds exhibit haemoagglutinating activity.[9-10,17-18] *Cassia nodosa* extract showed moderate antiplasmodial activity.[19] In Ghana, *Cassia nodosa* is used in preparations targeted towards the cure of malaria, constipation, cold, fever, gastric pain, and diabetes mellitus.[20] Some researchers reported anti-inflammatory, antioxidant and toxicity studies of the plant extract.[21] Thus literature survey indicated that these plants are less explored for medicinal potential.

Both these cultivated plants are quite similar in many ways and their taxonomic information is meager. Hence it is essential to carry out systematic studies on various aspects. The present work intended to find out distinguishing features between *Cassia javanica* and *Cassia nodosa*. Therefore the study involved macroscopy, microscopy, phytochemical screening, physicochemical studies and TLC analyses of secondary metabolites. The significant observations obtained through this work may prove their applicability in proper identification of plants. The same information can be highly useful in quality control of crude drug obtained from both plants.

MATERIALS AND METHODS

The present investigation involves the studies of authentic samples of *Cassia javanica* Linn. and *Cassia nodosa* Buch.–Ham. ex Roxb leaves. The leaf samples of *Cassia javanica* were obtained from Bangalore (Lal–Baug), Bhiwandi of

Thane district and Mumbai (Vile–Parle). The samples of *Cassia nodosa* were collected from Pune (Deccan). The matured leaves were collected during their flowering season of May to August. The botanical identity of the collected samples was confirmed using the standard herbaria at Botanical Survey of India (BSI), Pune and Blatter Herbarium of St. Xavier's College, Mumbai. The accession numbers of *Cassia javanica* Linn. and *Cassia nodosa* Buch.–Ham. ex Roxb. were BSI Kumavat-1, Blat. 15780 respectively.

For microscopic inspection transverse sections of lamina and midrib were used. The hand cut sections of leaf samples were stained with suitable stains and made permanent.[22-23] Quantitative microscopic study involved determination of stomatal number, stomatal index, palisade ratio, vein islet number and vein termination number. It was performed with the help of portions of whole leaf cleared in chloral hydrate and surface preparations of epidermii.[24-26]

For physicochemical analysis, determination of ash values and extractive values were done as per the standard procedures.[26-30] In qualitative phytochemical screening, known quantities of dried powders were subjected to cold maceration using water, ethanol and chloroform as solvents. The obtained concentrated extracts were further tested for different chemical constituents.[31-36]

In quantitative phytochemical evaluation, certain important secondary metabolites were estimated using dried pounded samples of the drugs. For saponins, initially the frothing property of an aqueous decoction of plant materials was measured in terms of foaming index.[25,29] Later saponin was extracted and estimated.[37] Similarly, quantification of other biomolecules viz., alkaloids, anthraquinone glycosides, flavonoids**,** phytosterols and tannins were done.[28,35,38-39] In quantitative phytochemistry, saponins and anthraquinone glycosides were differing significantly in two plants hence it was decided to perform TLC of these two secondary metabolites.

Thin Layer Chromatography (TLC) was performed on Silica Gel G plates using methanolic extracts of leaf powder. For saponins, 0.5 g powder of each drug was added in 50 ml of methanol separately and extraction was carried out in Soxhlet apparatus. The concentrated brown extract of both drugs were used along with a solvent system containing chloroform-glacial acetic acid-methanol-water (60:32:12:8) followed by spraying with Anisaldehyde-sulphuric acid reagent. Yellow to brown spots were observed.[40,41]

For anthraquinone glycosides, 5 g powder of each drug was added to 25ml 50% methanol and heated gently to warm solution, the extract was filtered and concentrated to use for loading. A solvent system of n-propanol-ethyl acetate-water (40:40:30) was utilized. Treatment of spraying agent with concentrated HNO_3 was followed by heating at 120°C for 15 minutes. Later plates were cooled and sprayed with 10% ethanolic KOH. Brown to yellow spots were observed in visible light.[40,41]

RESULTS

Macroscopical characters of any plant helps in preliminary identification. Morphologically, both the plants (*Cassia javanica* - **CJ** *and Cassia nodosa* - **CN**) are resembling to a great extent but few distinct features, such as canopy, knotted bark, colour of sepals differs slightly. According to Mohammed, (2019), volatile oil obtained from flowers of CJ acts against oral pathogen, the comparison given in Figure 1 would be of help in confirming identity in such experimentation.[42] The observations of macroscopy of leaflets are depicted in Table 1.

Figure 1: Sepal colour - Red in CJ and Green in CN.

Table 1: Macroscopy of leaflets.

Parameters	**CJ**	**CN**
Colour	Dark green above pale green below	Dark green above pale green below
Phyllotaxy	Opposite	Opposite
Length (cm) Breadth (cm)	3.5 - 4.0 - 4.5 1.8 - 1.9 - 2.1	2.3 - 4.0 - 4.5 1.1 - 1.3 - 1.6
Shape	Oblong	Oblong-lanceolate
Petiolule	Sub sessile	Sub sessile
Base	Obtuse, oblique	Obtuse,oblique
Margin	Entire	Entire
Apex	Mucronulate	Mucronulate
Upper surface Lower surface	Less pubescent More pubescent	Less pubescent More pubescent
Venation	Unicostate reticulate	Unicostate reticulate
Texture	Papery	Papery

In microscopy, transverse sections of both type of leaflets showed similar internal features such as upper smooth epidermis and lower sub-papillose epidermis, 2 layered palisade tissue, 4-5 layers of spongy tissue, tannin filled cells and poorly developed vascular tissues in lamina. In midrib portion, 2 layers of collenchyma and 3 layers of chlorenchyma were present below upper epidermis. The pericyclic sclerenchyma is of 7-8 layers and section showed starch grains, prismatic as well as rosette shaped calcium oxalate crystals and diosmin content. Both plants differ slightly in leaflet constants. The observations of microscopy of leaflets are depicted in **Table 2.**

Table 2: Results of Microscopic Constants.

Microscopic Constants	**Ranges**	
	CJ	**CN**
Type of Stomata	Anomocytic	Anomocytic
No. of Stomata -Lower Epidermis	440 - 453 - 480	440 - 473 - 490
Stomatal Index - Lower Epidermis	11.5 - 14.6 - 15.8	11.4 - 14.9 - 16.3
Palisade Ratio	1 : 5	1 : 4
Vein Islet No.	12 - 18 - 22	16 - 20 - 24
Vein Termination No.	9 - 17 - 23	12 - 16 - 19

The physicochemical characteristics of the drugs are studied with the aid of ash as well as extractive value. Ash values are indicative of mainly the inorganic content. The different composition of each drug is responsible for such variable constants. Similarly, the obtained extractive values are also distinctly different. The amount of water soluble content of is greater in CJ. The maximum alcohol extractable matter is in CN as compared to CJ.As saponin test is found positive in qualitative analysis, foaming index was determined for each drug. It gives an approximate idea about frothing ability

of the drug. These significant values are indicative of high content of saponins in the drugs. The observations of Physicochemical analysis are depicted in Table 3.

Table 3: Results of Physicochemical Analysis.

Ash Values % (w/w not > than)	**CJ** (Mean ± SD)	**CN** (Mean ± SD)
Total ash	**8.01 ± 0.25**	**6.32 ± 0.13**
Acid insoluble ash	2.11 ± 0.18	1.05 ± 0.67
Water soluble ash	1.01 ± 0.23	1.07 ± 0.54
Extractive values % (w/w not < than)	**CJ**	**CN**
Water extractive	**13.14 ± 0.25**	**11.52 ± 0.31**
Alcohol extractive	**16.80 ± 0.41**	**18.59 ± 0.29**
Chloroform extractive	**1.95 ± 0.37**	**1.6 ± 0.21**
Foaming Index	**166.66 ± 0.20**	**100 ± 0.12**

CJ and CN are phytochemically similar and show diverse types of secondary metabolites which can be of therapeutic interest. The observations of Qualitative Phytochemical analysis are depicted in Table 4.

Table 4: Results of Preliminary Phytochemical Screening.

Plant constituent test	**CJ**			**CN**		
	W	**E**	**C**	**W**	**E**	**C**
Test for Alkaloids	+	+	+	+	+	+
Test for Tannins	+	+	-	+	+	-
Test for Cardiac glycosides	+	+	+	+	+	+
Test for Cyanogenetic glycosides	+	+	+	+	+	+
Test for Anthraquinone glycosides	+	+	+	+	+	+
Test for Steroids	-	+	+	-	+	+
Test for Sterols	+	+	-	+	+	-
Test for Terpenoids	-	+	-	-	+	-
Test for Flavonoids	-	+	-	-	+	-
Test for Saponins	+	-	-	+	-	-

(Key - W - Water Extract, E - Ethanol Extract, C - Chloroform Extract, '+' - Present, '-' Absent)

Quantitative phytochemistry shows different relative share of each biomolecule in two drugs. In quantification of secondary metabolites, saponins are found to be in greater amount. The amount of alkaloids is also significant, anthraquinone glycosides are the main characteristic compound of genus *Cassia* Linn. They are comparatively greater in CJ than that of CN. Another important phytochemica such as flavonoid, sterols and tannins were found in more or less similar concentration in CJ and CN. The observations of Quantitative Phytochemical analysis are depicted in Table 5.

Table 5: Results of Quantitative Phytochemistry.

Phytochemicals	**CJ** (Mean % Value ± SD)	**CN** (Mean % Value ± SD)
Total Saponins	9.88% ± 0.34	9.52% ± 0.44
Total Alkaloids	5.03% ± 0.27	4.08% ± 0.12
Anthraquinone glycosides	**6.03% ± 0.19**	**0.945% ± 0.31**
Flavonoids	1.46% ± 0.20	1.36% ± 0.17
Total Phytosterols	0.64% ± 0.35	1.37% ± 0.27
Total Tannins	0.21% ± 0.25	0.29% ± 0.27

TLC analyses of secondary metabolites exhibited significant difference in numbers of components separated and their respective Rf values in case of saponins and anthraquinone glycosides. These values can be of great importance in chemo-taxonomic evaluation. Similarly it can act as quick identification key for differentiating CJ from CN. The observations of TLC analyses are depicted in Table 4.

Table 4: TLC Analyses of Phyto-constitutents.

Secondary metabolites	No. of components separated		Rf values	
	CJ	CN	CJ	CN
Alkaloids	4	4	I–0.82, II–0.76, III–0.58, IV–0.42	I–0.82, II–0.73, III–0.60, IV–0.44
Anthraquinone glycosides	**2**	**1**	**I–0.37, II–0.24**	**I–0.36**
Saponins	**4**	**2**	**I–0.63, II–0.58, III–0.45, IV–0.31**	**I–0.67, II–0.56**
Flavonoids	1	1	I–0.90	I–0.91
Sterols	1	1	I–0.72	I–0.71

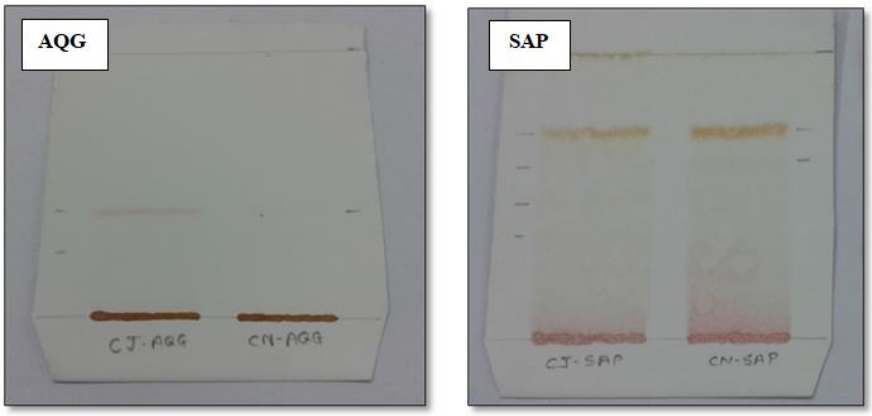

Figure 2: TLC plates of Anthraquinone glycosides (AQG) and Saponin (SAP).

CONCLUSION

The present work is an attempt to evaluate two ornamental species of *Cassia* with scientific approach. It deals with two aesthetic plants namely, *Cassia javanica* Linn. and *Cassia nodosa* Buch.-Ham. ex Roxb. According to the previous literature, apart from ornamental use, the above mentioned plants are known for their economic and medicinal claims. Recently two plants are being studied for novel therapeutic properties. In spite of belonging to same genus, these plants vary in medicinal actions. E.g. In preclinical antidiabetic studies on Wistar albino rats CJ (6.12%) showed less glucose reduction capacity compared to CN (9.03%). In herbal industry crude drug identity is utmost priority as it directly affects quality of final drug preparation. The said two species of pink cassias are difficult to differentiate in field using morphological characters. Leaflet constants, extractive values, quantitative estimation and TLC analysis of anthraquinone glycosides are the key identifying factors which can confirm identity of both plants efficiently. Through the present work, detailed pharmacopoeial standards and identifying keys are laid down for first time. These standards would prove to be of immense importance in analyzing the authentic quality drugs.

CONFLICTS OF INTEREST

The authors declare that there are no conflict of interests. The authors alone are responsible for the content of the paper.

AUTHORS' CONTRIBUTIONS

Both the authors were equally involved in designing research problem, collection of material and pharmacognostic - phytochemical evaluation of two allied species of pink Cassias. Manuscript is also prepared by consulting each other.

ACKNOWLEDGMENT

The authors are thankful to the Principal, Head - Department of Botany and Library of VPM's B.N.Bandodkar College of Science (Autonomous), Thane and SVKM'S, Mithibai College of Arts, Chauhan Institute of Science & Amrutben Jivanlal College of Commerce and Economics for supporting this research.

REFERENCES

1. Khurm M, Wang X, Zhang H, Hussain SN, Qaisar MN, Hayat K, Saqib F, Zhang X, Zhan G, Guo Z. The genus Cassia L.: Ethnopharmacological and phytochemical overview. *Phytotherapy research: PTR*, 2021; 35(5): 2336–2385.
2. Yadav SR, Sardesai MM. Flora of Kolhapur District. Kolhapur: Shivaji University - Kolhapur, 2002; 171-172.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants. Pune: Oriental Enterprises, 2001; 4: 1216-1219, 1226-1227.
4. Almeida MR. Flora of Maharashtra. 1st ed. Mumbai: Orient Press, 1998; 1: 182, 184-185.
5. Blatter E, Millard WS. Some Beautiful Indian Trees - Bombay Natural History Society. 2nd ed. Bombay: Oxford University Press, 1997; 26-31.
6. Haleem MA, Ul-Ain Q, Saadiq M, Iqbal M, Gulab H, Ali S, Khan S. Probing the chemical constituents of *Cassia javanica* and its *in vitro* analyses as a potent drug. *Royal Society open science*, 2022; 9(1): 211626.
7. Kapoor VP, Pandey K, Khanna M, Dwivedi AK, Singh S. Pharmaceutical applications of the galactomannan from the seeds of *Cassia javanica* Linn. *Trends in Carbohydrate Chemistry*, 1999; 5: 61-69.
8. Shyam, AK. Monographic and Floristic studies on the family Caesalpinaceae from India. Ph.D.Thesis, University of Pune, Maharashtra, India, 1997.
9. Anonymous. The Wealth of India - Raw Materials, New Delhi: Publication and Information Directorate CSIR, 1992; 3: 327-372.
10. Samant DD. Anatomical studies in genus *Cassia* Linn. Ph.D. Thesis, University of Mumbai, Maharashtra, India, 1985.
11. Satyavati GV, Raina MK, Sharma M. Medicinal Plants of India, New Delhi: Published by Indian Council of Medicinal Research, 1976; 1: 199-207.
12. Cheng H, Yang C, Lin T, Shieh D. Lin C. ent-Epiafzelechin-(4 alpha→8)-epiafzelechin extracted from *Cassia javanica* inhibits *Herpes simplex* virus type 2 replication. *Journal of Medical Microbiology*, 2006; 55(pt2): 201-206.
13. Kumavat UC, Jagdale SP, Shimpi SN. Hypoglycemic activity of *Cassia javanica* Linn. in normal and streptozotocin-induced diabetic rats. *Journal of advanced pharmaceutical technology & research*, 2012; 3(1): 47–51.
14. Kumar R, Ilyas M, Parveen M, Shafiullah. A new chromone from *Cassia nodosa*, *J Asian Nat Prod Res*, 2006; 8(7): 595-598.
15. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants, Lucknow & National Institute of Science Communication, New Delhi: Central Drug Research Institute, 1998; 5: 175-176.
16. Rai PP. Rhein from the leaves of *Cassia nodosa*. *Current Science*, 1979; 48: 15.

17. Roy B, Halder AC, Pal DC. Plants for human consumption in India. Calcutta: The Director, Botanical Survey of India, 1998; 41-42.
18. Randhawa GS, Mukhopadhyay A. Floriculture in India. Lucknow: Allied Publishers Limited, 1996; 212- 213.
19. Gameli A, Michael KL, Lawrence SB. Biological Efficacy and Toxicological Evaluation of Ethanolic Extract of Cassia nodosa Buch.-Ham. (Leguminosae). Journal of Chemistry, 2020; Article ID 3983491: 13.
20. Kumavat UC, Jagdale SP, Shimpi SN. Evaluation of hypoglycaemic activity of *Cassia nodosa* leaves in normal and streptozotocin-induced diabetic rats. *International Journal of Green Pharmacy*, 2012; 6(1): 9-13.
21. Mehta P, Parmar PH, Vadia SH, Patel MK, Tripathi CB. *In-vitro* antioxidant and *in-vivo* anti-inflammatory activities of aerial parts of Cassia species. *Arabian Journal of Chemistry*, 2017; 10: S1654–S1662.
22. Khandelwal KR. Practical Pharmacognosy. 12th ed. Pune: Nirali Prakashan, 2004.
23. Ruzin SE. Plant Microtechnique & Microscopy. USA: Oxford University Press, 1999.
24. Evans WC. Trease & Evans Pharmacognosy. 15th ed. London: W.B. Saunders Company Limited, 2002.
25. Mukherjee PK. Quality Control of Herbal Drugs - An approach of evaluation of Botanicals. 1st ed. New Delhi: Business Horizons, 2002.
26. Anonymous. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. 1. New Delhi: Government of India, Ministry of Health & Family welfare, Dept. of Indian systems of medicine & homoeopathy, 2001.
27. Anonymous. Indian Herbal Pharmacopoeia. Revised New Edition 2002. Mumbai: Indian Drug Manufacturer's Association, 2002.
28. Kulkarni PH, Apte BK. Research Methodology for Students of Ayurveda. Pune: Ayurveda Research Institute, 2000.
29. Anonymous. Quality control methods for medicinal plant materials. Geneva: World Health Organization, 1998.
30. Anonymous. The International Pharmacopoeia. Part-I. Vol. 1. 3rd ed. Geneva: WHO. CBS Publishers & Distributors, 1991.
31. Bindu G. Pharmacognosy and Pharmacological studies on some members of family Sapotaceae, Ph.D. Thesis. University of Mumbai, Maharashtra, India, (2009).
32. Singh GK, Bhandari A. Textbook of Pharmacognosy. New Delhi: CBS Publishers and Distributors, 2006.
33. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical Constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 2005; 4(7): 685-688.
34. Buzarbarua A. A textbook of Practical Plant Chemistry. 1st ed. New Delhi: S. Chand & Company Ltd, 2000.
35. Harborne JB. Phytochemical methods - A guide to modern techniques of plant analysis. 3rd ed. New Delhi: Springer (India) Pvt. Ltd, 1998.
36. Dickson C. Medicinal Chemistry Laboratory Manual, Investigations in Biological & Pharmaceutical Chemistry. Boca Raton USA: CRC Press LLC, 1999.
37. Rajpal V. Standardization of Botanicals – Testing and Extraction Methods of Medicinal Herbs. Vol. 1. New Delhi: Eastern Publications, 2001.
38. Kosalec I, Bakmaz M, Pepeljnjak S. Vladimir-Knezevic S. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharm*, 2004; 54: 65-72.
39. Goad L, Akihisa T. Analysis of Sterols. 1st ed. London: Blackie Academic and Professional, 1997.
40. Kowalska T, Sajewicz M. Thin-Layer Chromatography (TLC) in the Screening of Botanicals-Its Versatile Potential and Selected Applications. *Molecules (Basel, Switzerland)*, 2022; 27(19): 6607.

41. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Berlin: Springer-Verlag, 1996; 54-71, 305-327.
42. Mohammed HA, Abdel-Aziz, MM, Hegazy, MM. Anti-oral pathogens of *Tecoma stans* (L.) and *Cassia javanica* (L.) flower volatile oils in comparison with chlorhexidine in accordance with their folk medicinal uses. *Medicina*, 2019; 55(6): 301.