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Pharmacognostic evaluation of selected species of *Caralluma* genus

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Abstract

Objective: To establish a set of standardized parameters this will assist in identification of raw material of *Caralluma* species. **Materials:** The present study reports, detailed set of parameters from four species, *Caralluma lasiantha*, *Caralluma umbellata*, *Caralluma attenuata* and *Caralluma diffusa*, for powder characteristics, physicochemical evaluation, HPTLC fingerprint profile, and quantitative estimation of phytoconstituents that would contribute in the correct identity of the raw materials. **Results:** Various prominent cellular components were observed under the microscopic study, extractive values showed the presence of more water soluble compounds and phytochemical analysis revealed the presence nearly eight classes of phytoconstituents. HPTLC analysis showed the marked differences among species. **Conclusion:** The results of the present study serve as valuable information for correct identification of plant and determine its genuinity.

Keywords: *Caralluma*, Adulteration, HPTLC and physicochemical.

Introduction

The past decade has experienced a great paradigm shift towards the use of herbal products in modern system of medicine, this increasing popularity in herbal products has opened up new dimensions in the area of natural products for the greater demand in international market. The global market is estimated at 117 billion US dollar of which India's share is meager 0.9%, even though India has rich diversity of medicinal plants and their medicinal usage are well documented in folk and other Indian systems of medicine.[1] Hence, there is huge export opportunity for India's herbal industry; however this quest is full of challenges meeting global requirements of quality, safety, and efficacy of medicinal plants and herbal products.[2] Adulteration and substitution are major concern which may results into inconsistent quality, poor efficacy and safety.[3] Hence quality has to be assured at all stages – herbal raw materials collection, processing and finished herbal medicines. The major portion of adulteration can be controlled if standard practices are followed during the stage of collection of raw material and their identification i.e., by employing the standard protocols during collection of raw materials followed by testing them for their unique phytochemical profile to confirm their identity. These practices will give a definite idea on the quality of raw material in regard to adulteration and substitution. Plenty of standard techniques and protocols available for the well established medicinal plants and their raw materials. But in case of newly discovered medicinal plants and other plants which are less explored does not have standard collection and identification procedures, so in case of research involving new medicinal plant it is mandatory practice to standardize the plant materials along with their efficacy evaluation. In the present study we tried to standardize the plants belong to the genus *Caralluma* as part of our research work.

The genus *Caralluma* belongs to sub family Asclepiadaceae comprises about 200 species distributed throughout Asia and Africa, majority of these species are indigenous to the Indian sub continent and Arabian Peninsula.[4] *Caralluma* species have been used as a food for centuries in semi arid region of Pakistan and tribal people in India, and believed to reduce obesity and diabetes.[5] *Caralluma* species contain many bioactive principles, especially glycosides. The glycosides from *Caralluma* belong to pregnane group, these pregnane glycosides and other phytoconstituents have been reported for various biological activities viz., inflammation, rheumatism, diabetes, leprosy, antipyretic, anti-helminthetic activities.[6] The selected species *Caralluma lasiantha* (Wight) N.E.Br., *Caralluma attenuta* Wight, *Caralluma umbellata* Haw. and *Caralluma diffusa* (Wight) N.E.Br., have not been mentioned in any of the literature in regarding to their pharmacognostic features. Even earlier observations indicate that identification of different species from *Caralluma* genus is a great difficulty due to their more intermediate forms in their habitat.[7] Hence an attempt has been made to study a set of pharmacognostic parameters which will help in their identification, and may partly contribute to lay down their monograph.

Materials and Methods

Collection of plant materials

The Plant materials were collected from different locations of southern India during the month April 2011. *C. lasiantha* (Gooty hills, Andhra Pradesh. India), *C. attenuata*, *C. umbellata* (Tirupathi, Andhra Pradesh. India) and *C. diffusa* (Chitradurga, Karnataka. India). The plant materials were authenticated by Dr. Madava Chetty, Asst Professor, SV University, Tirupathi and were confirmed by comparing with the housed authenticated specimens.

Powder microscopic characteristics

The powdered raw materials were studied for the presence of various prominent cellular components. The samples from the whole aerial parts were subjected for microscopic analysis where the powder materials were treated with phloroglucinol followed by HCL and toludine blue.[8, 9]

Physico chemical parameters

Analysis of physico chemical parameters will help in judging out nature of raw material, parameters such as moisture content was studied to know the stability of raw material against degradation by microorganisms, pH was determined from 1% w/v suspension in distilled water by using digital glass electrode and extractive values of raw materials were studied to find out the extractable constituents in alcohol and water, different ash values were studied as per standard procedures.[10]

Fluorescence analysis

Fluorescence analysis of the powdered materials was performed to know the fluorescent patterns of samples at different wavelength of light after treatment with chemical reagents as per standard procedures.[11]

Preliminary phytochemical analysis

The powdered raw materials were extracted using a soxhlet apparatus with methanol (M), aqueous (A) and hydro methanolic (60:40, H) solvents. The obtained extracts were analyzed for the presence or absence of various classes of phytochemicals.[12]

HPTLC study

A qualitative densitometric HPTLC analysis was performed for the development of the characteristic fingerprint profile of hydro methanolic (60:40) extracts. The mobile phase, chloroform: methanol: water (6.5:3.5:0.5) was fixed to develop the chromatogram, after trying with various different system of mobile phases. The developed chromatogram was scanned at 254nm and 366nm and sprayed with vanillin sulphuric acid reagent.

Estimation of total phenols, tannins and flavonoids

The powdered materials were subjected for the estimation of total phenols and tannins by Folin ciocaltateu method.[13] Similarly flavonoid estimation was performed by vanillin sulphuric acid method.[14]

Results

Powder microscopic characteristics

The results of the powder study showed the presences of various cellular prominent structures, are described in Fig 1-4.

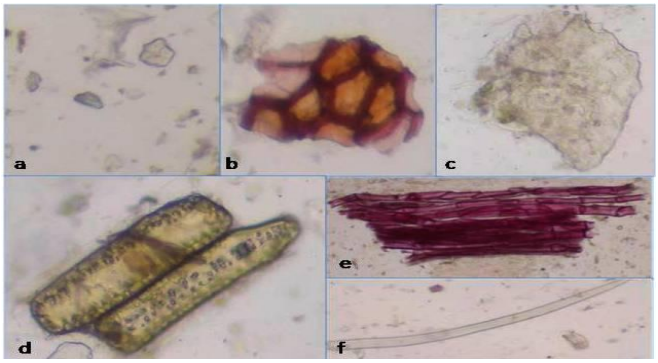


Figure 1: Powder characteristics of *C. lasiantha*. **a.** Calcium oxalte crystals, **b.** Parenchyma cells**, c.** Stone cells, **d.** Xylem vessels, **e.** Pholem vessels, **f.** Trichome

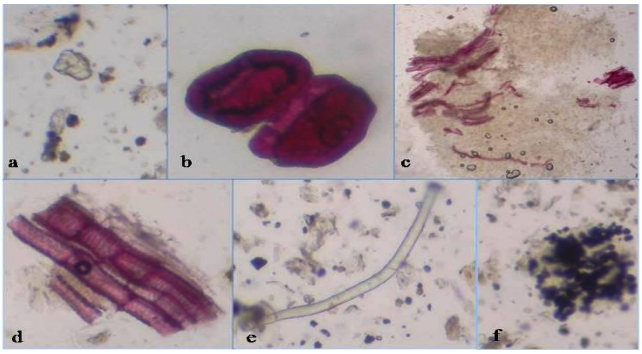


Figure 2: Powder characteristics of *C. umbellata*. **a.** Calcium oxalte crystals, **b.** Corticla Parenchyma cells, **c.** Fibrous cells lignified walls, **d.** Pholem vessels, **e.** Trichome, **f.** Starch grains associated with parenchyma cells.

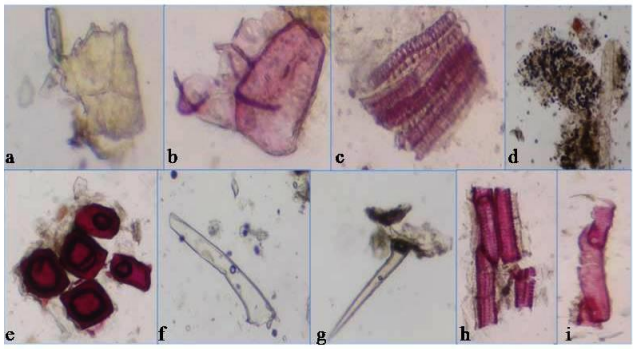


Figure 3: Powder characteristics of *C. diffusa*. **a.** Calcium oxalte crystals, **b.** Parenchyma cells, **c.** Phoelm rays, d. Starch granules, **e.** Stone cells, **f.** Unicellular epidermal trichome, **g.** Unicellular epidermal trichome intact, **h.** Xylem tissue with plate like opening, **i.** Vascular tissue.

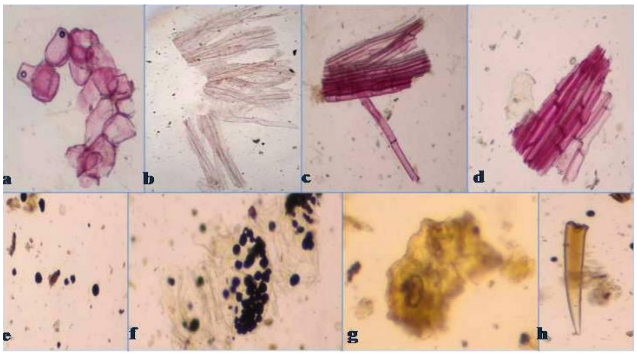
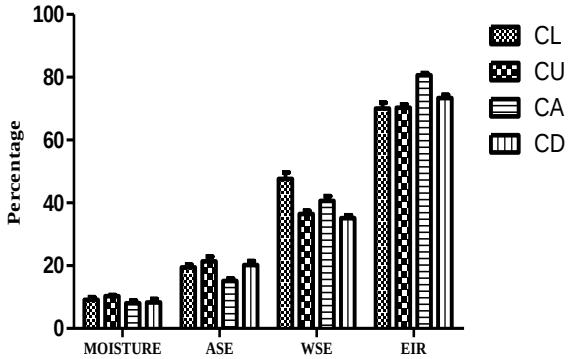


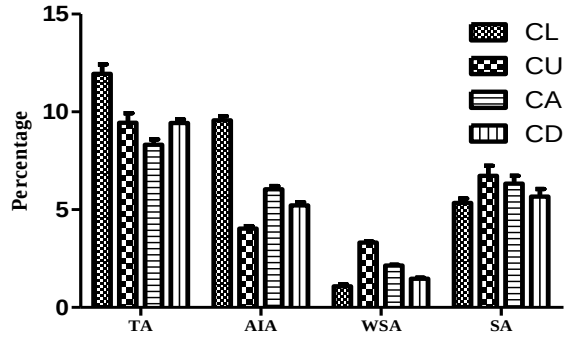
Figure 4: Powder characteristics of *C. attenuata*. **a.** Corticle parenchyma cells, **b.** Lignified fibrous tissue, **c.** Pholem vessels, **d.** Pitted xylem vessels, **e.** Simple starch grains, **f.** Starch grains associated with parenchyma cells, **g.** Stone cells, **h.** Unicellular epidemal trichome

Physicochemical parameters

The results for physicochemical parameters are described in Fig 5. The moisture content of four species was found to be less than 11%, and pH of the dried materials was found to be in the acidic range of 5 to 6.8. From the extractive values, the raw materials were found to be extracted high in aqueous rather than in alcohol indicating high content of water soluble compounds. The percentage aqueous extractive value was found to be highest with 47.63±2.10 in *C. lasiantha* to the lowest in *C. attenuata* with 35.09±0.92%. The ash values indicate that *C. lasiantha* contains a high percentage of total ash and acid insoluble ash with 11.95±0.51 and 9.56±0.30%, respectively. Similarly, *C. umbellata* contains a high percentage of water soluble and sulfated ash with 3.32±0.08 and 6.73±0.37%, respectively among the tested species.



A)



B)

Figure 5: Physicochemical evaluation of *C. lasiantha* (CL), *C. umbellata* (CU), *C. attenuata* (CA) and *C. diffusa* (CD). **A).** Moisture content and extracting values (ASE-Alcohol soluble, WSE-water soluble and EIR-alcohol insoluble extractive values) in percentages. **B)** Ash values viz., total ash (TA), acid insoluble ash (AIA), water soluble ash (WSA) and sulfated ash (SA). The values represented are average of three experiments (n=3).

Fluorescence analysis

The fluorescence analysis with several reagents was observed at three different wavelengths of light viz., short wavelength at 254nm, long wavelength at 366nm and under day light. The observed colors are recorded in table 1A and B.

Preliminary phytochemical analysis

In phytochemical screening, nearly eight phytoconstituents were found to be present in the analyzed extracts. Among which, phenols, flavonoids, carbohydrates and glycosides were found to be present in all the three extracts, where as triterpenoids and sterols were present only in methanolic and hydro methanolic extracts. Alkaloids were found to be absent in all test extracts and saponins were found be positive only in hydro methanolic extracts (Table 2).

HPTLC study and quantative analysis for total phenols, tannins and flavonoids

The HPTLC fingerprint profile was obtained from all four species (Fig 6), under visible light, 11 major spots were observed after derivatization with Rf value ranging from 0.10 to 0.95. A green color band with an Rf value of 0.68 was observed in all the four species, similar green band at Rf 0.95 was observed only in three species except HCA. The bands purple and green corresponding to 0.86 and 0.95 showed the presence in HCL and HCU. A similar pattern was observed among HCD and HCL samples at Rf values 0.63 and 0.68.

The results obtained from quantative analysis are summarized in Fig 7. The values represented are average of three experiments (n=3).

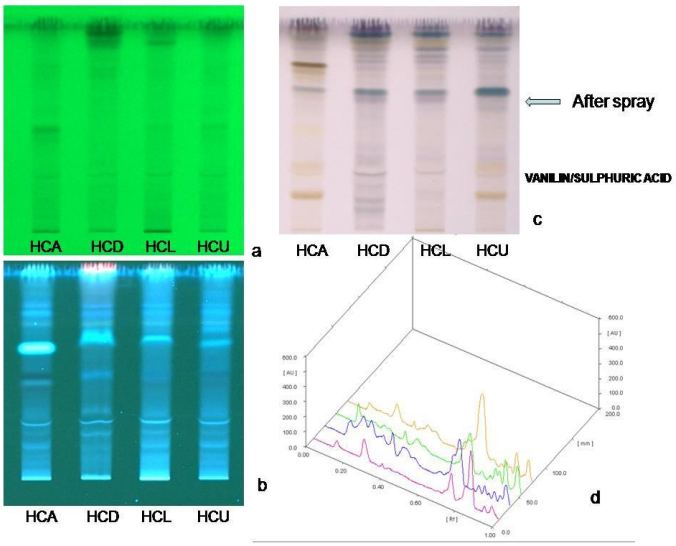


Figure 6: HPTLC fingerprint profile; HPTLC chromatogram visualized at 254 **(a)**, 366 **(b)** and 600 nm **(c)** showing a TLC pattern of hydro methanolic (H) extracts Pattern shown by 254 nm and 366 nm were recorded before derivatization while pattern shown by AS-600 nm was recorded at 600 nm after derivatization with vanillin sulfuric acid **(d)** HPTLC densitogram showing at 600 nm after derivatization.

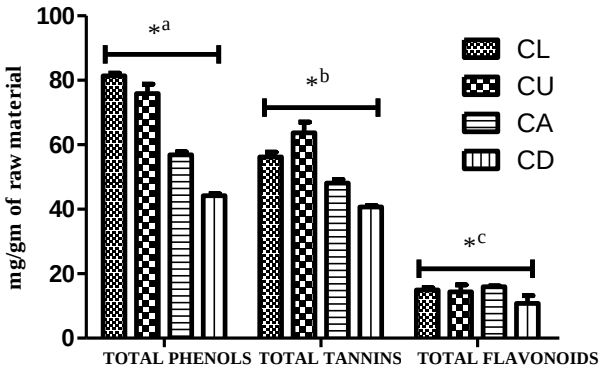


Figure 7: Quantative estimation of total phenols, tannins and flavonoids of *C. lasiantha* (CL), *C. umbellata* (CU), *C. attenuata* (CA) and *C. diffusa* (CD). *a, *b: values are expressed as gallic acid equivalent in mg/g of the raw material, *c: values are expressed as phloroglucinol equivalent in mg/g of the raw material. The values represented are average of three experiments (n=3).

Table 1.A: Fluorescence analysis of aerial part powder of *C. lasiantha* and *C. umbellata*

Treatment	*C. lasiantha*			*C. umbellata*		
	Day light	**L.W**	**S.W**	**Day light**	**L.W**	**S.W**
Powder (P) + water	Brown	Green	Green	Peech green	Green	Green
P + $CHCl_3$	Green	Orange green	Light green	Light green	Orange pink	Light Green
P + NaOH in H_2o	Brown	Green	Green	F. green	F. green	F. green
P + 1N NaOH in MeoH	Green	F. green	Light green	Green	F. green	F. green
P + 10% HCl	Light brown	Green	Light green	Cremish yellow	Green	Turbid Green
P + 10%H_2So_4	Yellowish brown	Green	Light green	Cremish yellow	Green	Turbid Green

L.W: Long wavelength (366 nm), S.W: Short wavelength (254 nm), F: Fluorescent

Table 1.B: Fluorescence analysis of aerial part powder of *C. diffusa* and *C. attenuata*

Treatment	*C. diffusa*			*C. attenuata*		
	Day light	**L.W**	**S.W**	**Day light**	**L.W**	**S.W**
Powder (P) + water	Brown	Green	Green	Dark brown	Green	Green
P + $CHCl_3$	Green	Orange green	Golden yellow	Green	F red	Green
P + NaOH in H_2o	Brown	Green	Green	Brown	Blackish violet	Blackish green
P + 1N NaOH in MeoH	Green	F. green	Golden yellow	Green	F.red	Blackish red
P + 10% HCl	Light brown	Green	Straw	Green	Green	Green
P + 10%H_2So_4	Light brown	Green	Straw	Green	Green	Green

L.W: Long wavelength (366 nm), S.W: Short wavelength (254 nm), F: Fluorescent

Table 2: Preliminary phytochemical analysis of extracts of studied *Caralluma* species

Test	MCL	ACL	HCL	MCD	ACD	HCD	MCU	ACU	HCU	MCA	ACA	HCA
Test for carbohydrates												
a. Molisch's test	+	+	+	+	+	+	+	+	+	+	+	+
Test for Glycosides												
a. Keller-Killiani test	+	+	+	+	+	+	+	+	+	+	+	+
Test for Saponins												
a. Foam test	-	-	+	-	-	+	-	-	+	-	-	+
Test for Alkaloids												
a. Mayer's test	-	-	-	-	-	-	-	-	-	-	-	-
b. Dragendrodroff's test	-	-	-	-	-	-	-	-	-	-	-	-
Test for Flavonoids												
Alkaline reagent test	+	+	+	+	+	+	+	+	+	+	+	+
Test for Phenolics and Tannins												
a. Ferric chloride test	+	+	+	+	+	+	+	+	+	+	+	+
b. Test for Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Test for Phytosterols and Triterpenoids												
a. Leiberman-Bucharat test	+	-	+	+	-	+	+	-	+	+	-	+
b. Salkowaski test	+	-	+	+	-	+	+	-	+	+	-	+
Test for fixed oils and fats												
a. Oily spot test	-	-	-	-	-	-	-	-	-	-	-	-

+ denotes presence of phytochemical, - denotes absence of Phytochemical

MCL: *C. lasiantha* alcoholic extract, ACL: *C. lasiantha* aqueous extract, HCL: *C. lasiantha* hydroalcoholic extract; MCD: *C. diffusa* alcoholic extract, ACD: *C. diffusa* aqueous extract, HCD: *C. diffusa* hydroalcoholic extract; MCU: *C. umbellata* alcoholic extract, ACU: *C. umbellata* aqueous extract, HCU: *C. umbellata* hydroalcoholic extract; MCA: *C. attenuata* alcoholic extract, ACA: *C. attenuata* aqueous extract, HCA: *C. attenuata* hydroalcoholic extract

Discussion

Caralluma species have been used traditionally around the world for various ailments viz., inflammation, rheumatism, diabetes, leprosy, antipyretic, anti-helminthetic activities. [16] As a first step in standardization, parameters such as time and place of collection of plant material too plays a crucial role in maintaining reproducibility in terms of phytochemical constituents and invariably biological activities of an extract. Thus present plant materials were collected from different locations based on previous published data.[15, 7] Microscopic analyses is one of the quickest methods for the correct identification of the source materials especially from the powdered materials.[16] Powder analyses showed the presence of various prominent parts of cellular components, parenchyma cells, cortical tissue, lignified fibrous tissue, phloem and xylem vessels, starch grains, stone cells, trichome, vascular tissue and calcium oxalate crystals were observed in all species. From the study although significant differences were not observed, however observed features may help to distinguish from the other substituted or contaminated material from insects, animal faeces or molds.

Various physico chemical parameters also used as a reliable data for raw material standardization, the extractive value gives us an idea about extent of adulteration and also nature of phytoconstituents present in the raw materials such as water soluble or alcohol soluble constituents.[17] From the study it is understood that all tested species contains more water soluble phytoconstituents such as glycosides, saponins and tannins rather than alcohol soluble constituents. These observations indicate caralluma species contains majorly the glycosides and saponins, which confirms the earlier report.[7] Ash values, which reflect the presence of carbonates, phosphates, oxides and also give an idea about earthy matter or inorganic composition and other impurities present along with the raw material.[18, 19] From the study minor differences was observed among the species, however the noted observations will enhance their specific identification of a specie and also gives us an idea on quality of raw material free from impurities.

Fluorescence analysis is an important analytical tool in the identification of herbal materials due to its rapidity and reproducibility. [19, 20] Fluorescence analysis showed marked differences among the species, different pattern of colors were observed. The observed colors thus can be used as indication tool about species geniuses. Phytochemical study showed the presence of similar class of phytoconstituents as reported earlier carbohydrates, phenols, flavonoids, tannins and glycosides found common in all species.[21, 22] Phytochemical profiling is an important and reliable parameter to understand the purity and genuiness of the raw material, any variation in their profile gives an idea of adulteration.

HPTLC fingerprint profile has a potential application in identification of a species in a rapid time. [23] HPTLC fingerprint revealed marked differences in their profile as displayed by differences in availability of bands. In visible light after derivatization, bands except at Rf value 0.68 and 0.95 the other bands do not match. Hence all species may be considered of having distant affinity from each other, which supports earlier study.[7] Hence the developed chromatogram may used to distinguish among the species and as well as authenticating the material.

Total phenols, tannins and flavonoid levels are important factors as a quality profile with respect to the therapeutic efficacy of plant; [24] estimation of these will give an idea on the adulteration of the raw material. So, in the present study we had estimated the levels of phenols, tannins and flavonoids which will help in understanding the purity of raw material.

Conclusion

Though the use herbs as a medicine has becoming popular even in the developed countries in recent days, one of the impediments in its widely acceptance is the lack of standard quality control profile, hence to overcome such drawback it is necessary to establish a quality profile of herbs through standardization of quality parameters and establishing the safety profile. The results of the present study are encouraging and may be used as a reference data towards monograph development.

References

1. Pandey M.M, Rastogi S, and Rawat A.K.S. Indian traditional Ayurvedic system of medicine and nutritional supplementation. Evidence-Based Complementary and Alternative Medicine. 2013; 12.

2. Nikam P.H, Kareparamban J, Jadhav A, Kadam V. Future trends in standardization of herbal drugs. Journal of Applied Pharmaceutical Science. 2012; 02: 38-44.

3. Ernst E. Heavy metals in traditional Indian remedies. Eur J Clin Pharmacol. 2002; 57:891-96.

4. Gilbert MG. A review of Caralluma R. Br. and its segregates. Bradleya. 1990; 8: 1-32.

5. Chandran R and Parimelazhagan T. Total Phenolic Content and Anti-Radical property of *Caralluma diffusa* (Wight) N.E. Br. Asian Pac J Trop Biomed. 2012; 1: 1-4.

6. Abdel-Satter E, Ahmed A.A, Mohamed Elamir FH Mohammed AF. Al-Yaha MA. Acylated pregnane glycoside from *Caralluma russeliana*. Phytochem. 2007; 68:1459-63.

7. Vajha M, Audipudi A.V, Murthy K.S.R. DNA Fingerprinting Profile of *Caralluma* Species: An Approach to Molecular Taxonomy. Asian J Phar Biol Res. 2011; 1:500-6.

8. Kokate C.K. Practical Pharmacognosy. New Delhi: Vallabh Prakashan; 1994.

9. Khandelwal K.R. Practical Pharmacognosy. 18th ed. Pune: Nirali Publication; 2007.

10. Government of India. Indian Pharmacopeia. 4th ed. New Delhi: Ministry of Health and Welfare, Controller of Publications; 1996.

11. Chase C.R, Pratt R.J. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharm Assoc. 1949; 38: 324-331.

12. Harbone J.B. Phytochemical Methods – A Guide to Modern Techniques of plant analysis. 2nd edition. Chapman and Hall London: New York; 1984.

13. Siddhuraju P, Becker K. Studies on antioxidant activities of Mucuna seed (*Mucuna pruriens* var. Utilis) extracts and certain non-protein amino/imino acids through in vitro models. J Sci Food Agric. 2003; 83: 1517-1524.

14. Swain T, Hillis WE. The phenolic constituents of *Purmus domestica*. I. The quantitative analysis of phenolic constituents. J Sci Food Agric. 1959; 10: 63-68.

15. Ramachandran VS, Thomas B, Sofiya C, Sasi R. Rediscovery of an endemic species, *Caralluma diffusa* (Wight) N.E. Br.(Asclepiadaceae) from Coimbatore District, Tamil Nadu, India after 160 years. J Threat Taxa. 2011; 3: 1622-1623.

16. Gupta P.C, Sharma.N, Rao C.V. Pharmacognostic studies of the leaves and stem of *Careya arborea* Roxb. Asian Pac J Trop Biomed. 2012:404-408.

17. Jahan N, Afaque NA, Ahamad G, Ansari A.A. Physico- chemical studies of the Gum Acacia. Indian J Nat Prod Resour. 2008; 7:335-337.

18. Nirmal S.A, Pal S.C, Mandal S.C. Pharmacognostic evaluation of *Nyctanthes arbortristis* bark. Asian Pac J Trop Biomed. 2012: S494-S500.

19. Muhammad N, Muhammad S, Ibrar B.M, Khan H. Pharmacognostic studies of *Viola betonicifolia*. Afr J Pharm Pharacol. 2012; 6(1):43-47.

20. Kokoshi C.J, Kokoshi R.J, Sharma P.J. Fluorescence of powdered vegetable drugs under UV radiation. J Am Pharm Assoc. 1958; 47: 715-717.

21. Kamil M, Fjayaraj A, Ahmad F, Gunasekhar C, Samuel S, Chan K, et al. Identification and quantification of flavonoids from *Caralluma arabica* and its quality control studies. J Pharm Pharmacol. 1999; 51:225.14.

22. Ramesh M, Nageshwara Rao Y, Rama Kumar M, Rao Appa, Prabhakar MC, Madhava Reddy B. Antinociceptive and anti-inflammatory activity of carumbelloside-I isolated from *Caralluma umbellata*. J. Ethnopharmacol.1999; 68:349-352.

23. Arunachalam K, Iniyavan M, Parimelazhagan T. A HPTLC Method for the Identification of Potential Therapeutic Compound of Kaempferol from *Ficus amplissima* Smith. Int. J. Pharm. Sci. Rev. Res. 2013; 22(1):166-171.

24. Wojdyło A, Oszmian´ski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007; 105:940–949.