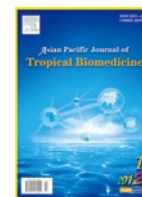




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading

Pharmacognostical and physico–chemical standardization of *Syzygium cumini* and *Azadirachta indica* seedP Bigoniya^{1*}, CS Singh¹, B Srivastava²¹Radharaman College of Pharmacy, Bhopal, Madhya Pradesh, India²School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India

ARTICLE INFO

Article history:

Received 14 December 2011

Received in revised form 2 January 2012

Accepted 28 February 2012

Available online 28 April 2012

Keywords:

*Syzygium cumini**Azadirachta indica*

Macroscopic characters

Microscopic characters

Physicochemical studies

Phytochemical studies

ABSTRACT

Objective: To perform pharmacognostical and physico–chemical standardization of seeds of *Syzygium cumini* Linn. (*S. cumini*) and *Azadirachta indica* A. Juss (*A. indica*). **Methods:** Fresh seed samples were studied macroscopically and microscopically. WHO recommended parameters were estimated for standardization of seeds. Preliminary phytochemical investigation and quantitative estimation of phytoconstituents was also performed. **Results:** The detail microscopy of *S. cumini* revealed the presence of single cotyledon consisting of single layered epidermis. Parenchymatous cells were fully packed with simple, oval, rounded starch grains. Transverse section of *A. indica* seed showed oil grains in endosperm. The epidermis was single layered attached to tegmen. The embryo was imbedded inside the endosperm and contained tigellum. Physicochemical parameters such as total ash, acid insoluble ash, loss on drying, percentage of foreign matters and extractive values were determined. Fluorescence analysis of both the seeds was determined in number of organic and inorganic solvents. Preliminary phytochemical screening of *S. cumini* and *A. indica* in different solvent showed the presence of alkaloid, carbohydrate, inulin, mucilage, starch, triterpenoid and flavanoid. *S. cumini* was found to contain high concentration of flavanoid (7.44%) and phenols (16.88%) whereas *A. indica* showed rich presence of saponin (2.78%) and phenol (2.42%). **Conclusions:** The pharmacognostical and physicochemical standards developed in this study will provide referential information for identification and standardization of *S. cumini* and *A. indica* crude drugs.

1. Introduction

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. In the western world, as the people are becoming aware of side effects of synthetic drugs, there is an increasing interest in the natural remedies with a basic approach towards the nature. WHO estimates that about 80% of people in developing countries still realize on traditional medicine based largely on plants and animals for their primary healthcare^[1]. Herbs are comparatively safe because of their low toxicities. Herbal medicines are currently in demand and their popularity is increasing day by day. In the healthcare sector, WHO recommends and encourages the use of traditional herbs/remedies because of easy availability and affordability.

However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed

countries, is the lack of proper documentation, stringent quality control and standardization. These problems arise from the complex composition of drugs which are used in the form of whole plant, parts of the plant(s) and of plant extracts. Standardization of the presumed active compounds of drug in general does not reflect reality. Only in a few cases drug activity depend upon single component. Generally, it is the result of concerted activity of several active compounds as well as of inert accompanying substances treating the particular disease. There is a growing concern for documentation of research work carried out on traditional medicines needed for regulatory control^[2].

With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material used for therapeutic purposes. The process of standardization can be achieved by stepwise pharmacognostic studies and minimizing the inherent variation of natural product composition through quality assurance practices applied to cultivation and manufacturing processes.

Keeping in view the above mentioned problems, an attempt has been made to standardize the ethnopharmacologically useful seeds of *Syzygium cumini* Linn. (*S. cumini*) and *Azadirachta indica* A. Juss (*A. indica*), commonly

*Corresponding author: Dr. Papiya Bigoniya, Principal, Radharaman College of Pharmacy, Bhabhada Road, Ratibad, Bhopal– 462 002, Madhya Pradesh, India.

Tel: +91–0755–2477941, +91–0755–2896237, +91–9827011258

Fax: +91–0755–2896664

E-mail: p_bigoniya2@hotmail.com

Foundation project: It is supported by Tapsya Siksha Samiti, Bhopal and laboratory facilities (Faculty Promotion/July–Dec 2010/rcp–02).

available and widely used in central India, based on pharmacognostical and physiochemical characteristics.

S. cumini (Fam. Myrtaceae) is commonly known as Jambu. This is tree attaining a height upto 30 m and a girth of 3.6 m with a bole up to 15 m, found throughout India. *S. cumini* has been reported to have hypoglycemic effects both in experimental models and clinical studies. The seeds have neuropsychopharmacological and anti-HIV activity. Seed kernel has reported to decrease the oxidative stress in diabetic rats, which may be responsible for its hypoglycemic property^[3].

A. indica (Fam. Meliaceae) is commonly known as Neem. This is evergreen tree attaining a height upto 15–20 m or more and found throughout the plains of India. The seeds are brownish and dorsally convex. Almost every part of the tree is being used to treat different human ailments and also regarded as a household pesticide. The extract of bark, leaves, fruits and roots has been used to treat leprosy, intestinal helminthiasis and respiratory disorders in children. The bark extract is used as tonic, astringent and useful in relieving fever, thirst, nausea, vomiting and skin diseases. Other reports on the biological and pharmacological studies showed antiviral, anti-inflammatory, antipyretic and antioxidant properties^[4].

2. Materials and methods

2.1. Chemicals

FAA solution (95% ethyl alcohol: glacial acetic acid: formalin: water in 50:5:10:35), hemalum, safranin, hydrochloric acid, phloroglucinol and other chemicals used in the study were of analytical grade.

2.2. Plant material

The fresh seeds of *S. cumini* and *A. indica* were collected from their natural habitat, surrounding Bhopal, Madhya Pradesh, India during September 2010. The seeds were identified by Dr. Madhuri Modak, Professor, Department of Botany, MVM College, Bhopal, India with voucher 1132.68/281 for *S. cumini* and 1121.44/121 for *A. indica*. The collected seeds were washed, shade dried and pulverized with mechanical pulverizer for size reduction. The size pulverized seed powder was passed through mesh 40–60 and used for determination of physiochemical parameters and preparation of different solvent extracts. The fresh seed samples were used for macroscopic and microscopic studies.

2.3. Macroscopic and microscopic analysis

The macroscopy of the seeds were studied according to the method of Brain *et al*^[5]. In microscopy, the fresh seeds were cut into pieces of 2–5 mm without compression and immediately transferred into FAA solution for one day to kill and fix the tissues. The pieces were embedded with paraffin wax. The paraffin embedded specimens were sectioned with the help of rotary microtome having thickness of 10–12 μ m. Dewaxing of the sections was performed by customary procedure^[6]. The sections were stained with hemalum and safranin. A drop of HCl and phloroglucinol were used to detect lignified cell in the cut sections^[7].

2.4. Preparation of extracts

Coarse powder (25 g) of each seed was defatted individually with 500 mL of petroleum ether (40–60°C) with the aid of Soxhlet apparatus for 24 hr. The defatted seed cakes (5 g each) were then extracted separately with 100 mL each of ethyl acetate, chloroform methanol, ethanol and water for 48 hr by maceration and then filtered to obtain respective extracts. The petroleum ether fraction obtained after defatting was recovered as petroleum ether extract after filtration. The extracts in different solvent were collected separately and volume reduced under low pressure. Twenty five ml of the each extract was used to determine the percentage extractive values of seeds in different solvents. The remaining extract was stored in air tight glass container at 4–8°C for fluorescence analysis.

2.5. Physico-chemical studies

The percentage of foreign matter, loss on drying, total ash and acid insoluble ash were determined according to the method described in WHO guidelines on quality control methods for medicinal plants materials^[8]. The dried seed powders were subjected to fluorescence analysis, as it is and also after treating separately with 1 N of HCl, HNO₃, H₂SO₄, NaOH, KOH, alcoholic NaOH, alcoholic KOH and ammonia against normal and ultra-violet light (254 nm). Color reaction of petroleum ether, ethyl acetate, chloroform, methanol and water extract was also observed in normal light and UV light (254 nm)^[9].

2.6. Preliminary phytochemical screening

Preliminary phytochemical screening of the seed extracts in different solvents has been performed to detect the phytoconstituents like; alkaloid, amino acid, carbohydrate, glycoside, inulin, mucilage, tannin, starch, saponin, steroid, triterpenoid and flavonoid^[10].

2.7. Quantitative estimation of phytoconstituents

2.7.1 Alkaloid estimation

Alkaloid estimation was performed according to the method described by Obdoni and Ochuko^[11].

2.7.2. Flavonoid estimation

Aluminium chloride colorimetric technique was used for flavonoids estimation^[12].

2.7.3. Saponin estimation

Saponin estimation was performed according to the method described by Obdoni and Ochuko^[11].

2.7.4. Estimation of total phenols

The total phenols of both the extracts were measured at 765 nm by Folin Ciocalteu reagent method^[12].

3. Results

3.1. Morphological evaluation of seeds

3.1.1. *S. cumini*

The seeds are brownish black in color which is

approximately 2 cm long and 1 cm wide. Whole seed of *S. cumini* is enclosed in a cream colored, coriaceous covering which is smooth, oval or sometimes roundish in shape. The seed is astringent in taste (Figure 1).

3.1.2. *A. indica*

The seeds are brownish in color, dorsally convex, upto 1.2 cm long and 0.5 cm wide. The seed coat of *A. indica* is thin, brown in color and form a shell like structure over the seed. The seed coat usually cracks on touch. The inside of cracked pieces appears as golden yellow and seed kernels are light brown in color. The seed is oily in odour and having bitter taste (Figure 2).

3.2. Microscopic evaluation of seeds

3.2.1. *S. cumini*

Seed showed single cotyledon consisting of single layered epidermis. Seed coat is thin single layered which usually got detached after drying. The major bulk of seed is endosperm which stores the food in form of starch grains. Parenchymatous cells are fully packed with simple, oval, rounded starch grains. Few schizogenous cavities are also present (Figure 3).

3.2.2. *A. indica*

Transversely cut surface of *A. indica* seed showed endosperm in which oil granules are present. The section

Table 1.

Extractive values of *S. cumini* and *A. indica* seed in different solvents.

Seeds	Extractive value in % w/w					
	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Alcohol	Water
<i>S. cumini</i>	0.244	0.488	0.540	0.266	7.960	18.560
<i>A. indica</i>	1.968	1.500	1.912	1.008	26.800	19.360

Table 2.

Fluorescence analysis of *S. cumini* and *A. indica* seed powder.

Seeds	Observation under	Powder + Solvents									
		Dry powder	Powder +Water	Powder +HCl	Powder +HNO ₃	Powder +H ₂ SO ₄	Powder +NaOH	Powder +KOH	Powder +Alc. NaOH	Powder +Alc. KOH	Powder +Ammonia
<i>S. cumini</i>	Normal light	Earthy white	Golden rod	Gold	Gold	Yellow	Brown	Sienna	Navajo white	Golden rod	Saddle brown
	U.V. light	Earthy brown	Lawn green	Lawn green	Lawn green	Lime green	Greenish brown	Greenish brown	Greenish yellow	Lawn green	Greenish brown
<i>A. indica</i>	Normal light	Yellowish brown	Gold	Dark golden rod	Yellow	Light golden rod	Sienna	Golden rod	Gold	Gold	Golden rod
	U.V. light	Khakhi	Greenish yellow	Light green	Lawn green	Greenish yellow	Brownish black	Olive green	Lawn green	Lawn green	Olive drab

Table 3.

Fluorescence analysis of *S. cumini* and *A. indica* seed with different solvents.

Seeds	Extract	Normal light	UV light
<i>S. cumini</i>	Petroleum ether	Light yellow	Greenish yellow
	Ethyl acetate	Golden rod	Lawn green
	Chloroform	Light yellow	Greenish yellow
	Methanol	Golden rod	Lawn green
<i>A. indica</i>	Petroleum ether	Cream	White smoke
	Ethyl acetate	Pale golden rod	Dark sea green
	Chloroform	Golden rod	Forest green
	Methanol	Gold	Greenish yellow

Table 4.

Preliminary phytochemical screening of *S. cumini* and *A. indica* seeds with different solvents.

Seeds	Solvent	Phytoconstituents											
		Alkaloid	Amino acid	Carbohydrate	Glycoside	Inulin	Mucilage	Tannin	Tannin	Saponin	Steroid	Triterpenoid	Flavanoid
<i>S. cumini</i>	Petroleum ether	-	-	+	-	-	-	-	+	-	-	-	-
	Chloroform	-	-	+	-	+	+	+	-	-	-	+	-
	Ethyl acetate	-	-	+	-	+	+	-	-	-	-	-	-
	Methanol	+	-	-	-	+	+	+	-	-	-	+	+
	Water	+	-	+	-	-	+	+	+	-	-	+	+
<i>A. indica</i>	Petroleum ether	-	+	-	-	+	+	-	-	-	+	-	-
	Chloroform	-	+	+	+	+	+	-	+	-	+	-	+
	Ethyl acetate	-	+	-	-	-	+	-	-	-	-	+	-
	Methanol	+	+	+	-	+	+	-	-	-	-	-	+
	Water	-	+	-	-	+	+	-	-	-	-	+	-

“+” = Presence of constituent, “-” = Absence of constituents

contains an outer yellowish brown hard layer called testa and inner tegmen. The epidermis is single layered which was attached to tegmen. The epidermis and endosperm was well separated. The endosperm is fleshy oil storage tissue under the cover of perisperm. The embryo is imbedded inside the endosperm and contain thick cotyledon to an axis called tigellum. The tigellum showed a protruding radical plumule hidden between the cotyledons (Figure 4).

3.3. Physico-chemical evaluation

Foreign matters were found to be 0.28% in *S. cumini* and 0.37% in *A. indica*. Loss on drying of both the seed turned out to be 1.650% in *S. cumini* and 4.769% in *A. indica*. Content of total ashes in seed powder were found to be 0.34% in *S. cumini* and 6.63% in *A. indica*. The result showed the low content of acid insoluble ash in *S. cumini* (0.144%) and *A. indica* (1.966%) seed. The results of extractive value in different solvents are tabulated in Table 1.

3.4. Fluorescence analysis of drug powder and extracts



Figure 1. *S. cumini* fruit and seed.



Figure 2. *A. indica* fruit and seed.

The result of fluorescence studies on seed powder using different reagents are given in Table 2 and that of the extracts is compiled in Table 3.

3.5. Preliminary phytochemical test for extracts

Results of preliminary phytochemical screening are compiled in Table 4.

3.6. Quantitative estimation of phytoconstituents

The results of quantitative estimation indicate that *S. cumini* seed have higher percentage yield of flavonoid (7.44%), saponin (3.36%) and total phenol (16.28%), whereas the percentage yields of alkaloid recorded were minimal (0.23%). The percentage yield of flavonoid, saponin and total phenol in *A. indica* was found to be 1.97%, 2.78% and 2.42%, respectively whereas yield of alkaloid was 0.715%.

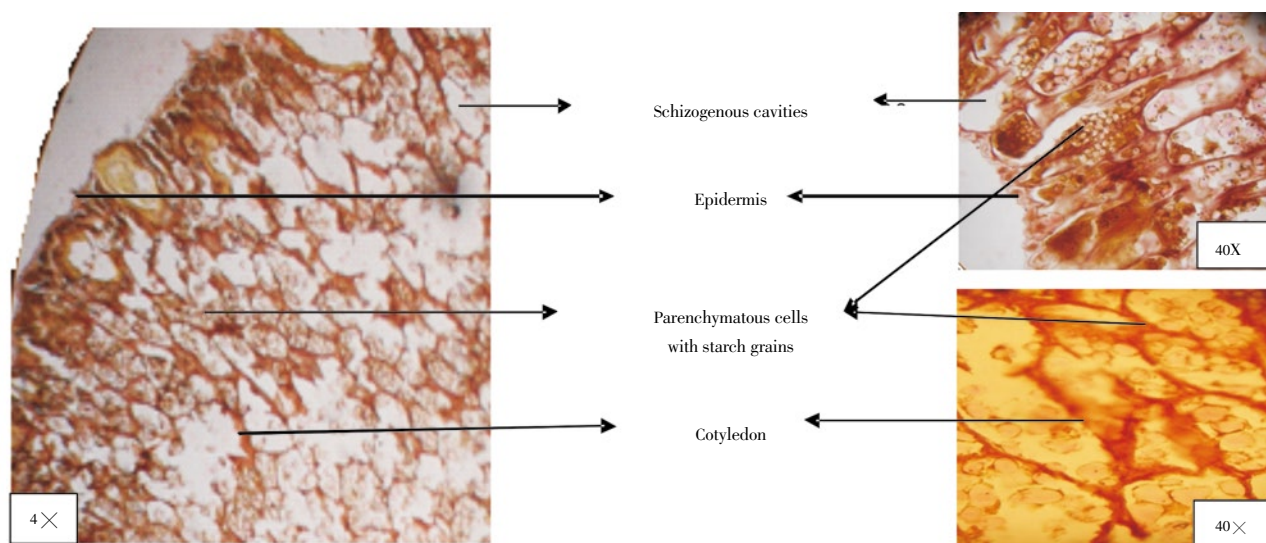


Figure 3. Transverse section of *S. cumini* seed.

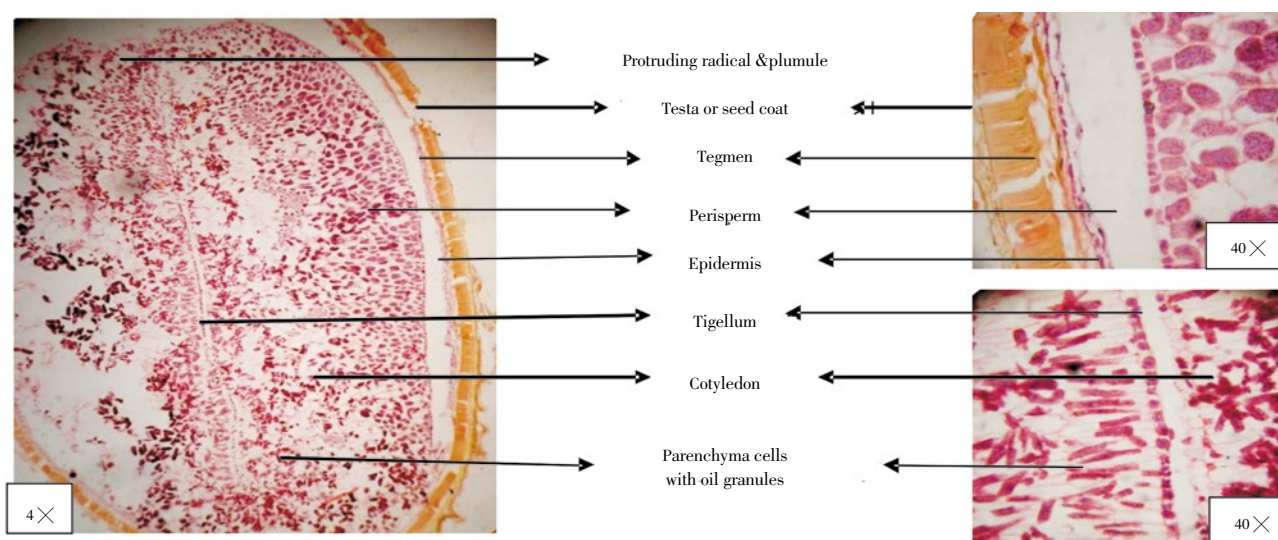


Figure 4. Transverse section of *A. indica* seed.

4. Discussion

Despite the availability of hyphenated analytical techniques, identification and evaluation of plant drugs by pharmacognostical and physico-chemical parameter study is still more reliable, accurate and inexpensive. According to World Health Organization (WHO), the macroscopic and microscopic determination of the plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken^[13]. In the present work the macroscopic and microscopic study of *S. cumini* and *A. indica* seeds were carried out. The results of macroscopic study might be useful for distinguishing it from its substitutes and adulterants. Microscopic evaluation allows more detailed examination of crude and enable to identify the organized structural features such as epidermis, starch grains in endosperm, parenchymatous cells and schizogenous cavities as present in *S. cumini* and oil granules in endosperm and tigellum in *A. indica*.

The physico-chemical parameters are helpful in judging the purity and quality of the drug. The foreign matters were present in negligible amount in both the seeds. This may be due to first hand collection of plant material from non polluted area^[14]. Loss on drying for *A. indica* was nearly

five percent. It signifies the considerable amount of moisture in seeds. The percentage of active chemical constituents in crude drugs is usually mentioned on air-dried basis. Hence, the moisture content of a drug should be determined and also should be controlled to make the solution of definite strength. The moisture content of a drug should be minimized in order to prevent decomposition of crude drug either due to chemical change or due to microbial contamination.

Ash values were used to detect the presence of any siliceous contamination and water soluble salts. These values are important quantitative standards as it is useful in determining authenticity and purity of drugs^[10]. Lower content of total ashes in the results indicate low level of carbonates, phosphates, silicates and silica in the seeds. The total ash value for a crude drug is not always reliable, since there is possibility of presence of non-physiological substances. So, the authentication of acid insoluble ash was also performed which showed low content of acid insoluble ash in both the seeds.

The results suggest that both the powdered seeds have high water soluble extractive value in comparison to the petroleum ether, chloroform, ethyl acetate, methanol and ethanol soluble extractive values. The water soluble

extractives indicate the presence of water soluble matters such as alkaloid, amino acids, carbohydrate, mucilage, triterpenoid and flavonoid derived from the seeds. These organic ligands possess promising biological activities, which can be utilized to develop potential drugs.

The results of fluorescence analysis of seed powders showed their characteristic fluorescent color in different organic and inorganic solvents. The fluorescence character of powdered drug plays a vital role in the determination of quality and purity of the drug material. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range of daylight. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which is not visible in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by treating with different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation^[15].

The results of preliminary phytochemical test showed the presence of various phytochemical compounds in the seeds which are known to have various therapeutic importance in medicinal sciences. For instance saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects. Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities^[16,17]. Rupasinghe *et al* have reported that saponins possess hypocholesterolemic and antidiabetic properties^[18]. The terpenoids have also been shown to decrease blood sugar level in animal studies. Steroids and triterpenoids showed the analgesic properties^[19]. The steroids and saponins are responsible for central nervous system activities^[20].

The seeds under study can be utilized as a potential source of useful therapeutics and the outcome data will be beneficial for quantitative and qualitative standardization of herbal preparations containing *S. cumini* and *A. indica* seed. Further studies are in progress on these seeds in order to isolate, identify, characterize and elucidate the structure of bioactive compounds along with exploration of their pharmacological activity.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors are thankful to Tapsya Siksha Samiti, Bhopal for providing financial support and laboratory facilities to carry out the research work (Faculty Promotion/July–Dec 2010/rcp–02).

References

- [1] Datta S, Ghosh A, Pal P, Das M, Kar PK. Pharmacognostical, phytochemical and biological evaluation of *Cardiospermum halicacabum*. *Int J Pharm Sci Bio* 2010; **1**(1): 37–42.
- [2] Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. *Ind J Pharmacol* 2000; **32**(4): 81–118.
- [3] Ayyanar M, Pandurangan SB. *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pac J Trop Biomed* 2012; 240–246.
- [4] Thani AM, Parcha V, Pant G, Dhulia I, Kumar D. *Azadirachta indica* (Neem) Leaf: A review. *J Pharm Res* 2011; **4**(6): 1824.
- [5] Brain KR, Turner TD. *The practical evaluation of phytopharmaceuticals*. Bristol: Wright–Scientechica; 1975.
- [6] Prabhu K, Karar PK, Ponnudurai K, Hemalatha S. Pharmacognostic and preliminary phytochemical investigations on the leaves of *Viburnum punctatum* Buch.–Ham.ex D.Don. *J Pharm Sci & Res* 2009; **1**(2): 43–50.
- [7] Das C, Das S, Sahoo DC. Evaluation of pharmacognostical and physico–chemical standard of the leaf of *Cassia tora* Linn. *Asian J Pharm Hea Sci* 2011; **1**(4): 216–220.
- [8] WHO. *Quality control methods for medicinal plant material*. Geneva: Organization Mondiale De La Sante; 1992.
- [9] Bigoniya P, Singh CS, Shukla A. Pharmacognostical and physicochemical standardization of ethnopharmacologically important seeds of *Lepidium sativum* Linn. and *Wrightia tinctoria* R. Br. *Indian J Nat Prod Resour* 2011; **2**(4): 464–471.
- [10] Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta states of Nigeria. *Global J Pure Appl Sci* 2001; **8b**: 203–208.
- [11] Mujeeb M, Siddique NA, Najmi AK, Akram M. Evaluation of antioxidant activity, quantitative estimation of phenols and flavonoids in different parts of *Aegle marmelos*. *African J Plant Sci* 2010; **4**(1): 1–5.
- [12] WHO. *Quality control methods for herbal materials*. Geneva: WHO Press; 2011.
- [13] Khandelwal KR. *Practical pharmacognosy, techniques and experiments*. 17th ed. Pune: Nirali Prakashan; 2007.
- [14] Kokate CK. *Practical pharmacognosy*. 12th ed. New Delhi: Vallabh Prakashan; 2008.
- [15] Mukherjee PK. *Quality control of herbal drugs: An approach to evaluation of botanicals*. New Delhi: Business Horizons; 2010.
- [16] Orhan I, Kupeli E, Sener B, Yesilada E. Appraisal of anti-inflammatory potential of the clubmoss, *Lycopodium clavatum* L.J. *Ethnopharmacol* 2007; **109**: 146–150.
- [17] Sharma B, Balomajumder C, Roy P. Hypoglycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin induced diabetic rats. *Food Chem Toxicol* 2008; **46**(7): 2376–2383.
- [18] Rupasinghe HP, Jackson CJ, Poysa V, Di Berado C, Bewley JD, Jenkinson J. Soyasapogenol A and B distribution in Soybean (*Glycine Max* L.Merr) in relation to seed physiology, genetic variability and growing location. *J Agric Food Chem* 2003; **51**: 5888–5894.
- [19] Srivastava M, Kumar A, Pal M. Phytochemical investigation on *Jatropha curcas* seed cake. *Int J Pharm Life Sci* 2010; **1**(6): 357–362.
- [20] Salna KP, Sreejith K, Uthiralingam M, Prince MA, John Milton MC, Fleming AT. Comparative study of phytochemicals investigation of *Andrographis paniculata* and *Murraya koenigii*. *Int J Pharm Pharm Sci* 2011; **3**(3) 291–292.
- [21] Srivastava M, Kumar A, Pal M. Phytochemical investigation on *Jatropha curcas* seed cake. *Int J Pharm Life Sci* 2010; **1**(6): 357–362.
- [22] Cocker JD, Elks J, May PJ, Nice FA, Phillipps GH, Wall WF. Action of some steroids on the central nervous system of the mouse. I. synthetic methods. *J Med Chem* 1965; **8**(4): 417–425.