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Research Article

Phytochemical & HPTLC analysis of roots of *Acacia arabica* Willd.

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Abstract: The present work has been undertaken to establish the necessary pharmacognostic standards and phytochemical constituents for evaluation of roots of *Acacia arabica* Willd (family Fabaceae). Plant is studied for its morphology, microscopy, phytochemicals present in plant root and HPTLC was carried out for quantification of quercetin in root extract. Various morphological parameters of fresh as well as shade dried form of roots were studied. Microscopy showed the presence of medullary rays, pith, phloem fiber, cork cells, epidermis and xylem cells in root. Physico-chemical constants such as fluorescence analysis of root powder and extracts, ash values, loss on drying, extractive value, swelling index, percentage extractive values of extracts with different solvents, consistency and color of different extracts under ordinary and UV light were studied. Phytochemical screening of total ethanolic and aqueous extracts showed the presence of flavonoids, tannins, saponin glycosides & reducing sugars. HPTLC was carried out for quantification of quercetin in ethanolic extract of the roots of *A. arabica* Willd. It was concluded that the *Acacia Arabica* Willd contains various phytochemicals; among these tannins and flavonoids are its main constituents.
Keywords: *Acacia arabica* Willd., Pharmacognostic, Phytochemical, Microscopy, HPTLC

INTRODUCTION

Acacia arabica Willd. *or Acacia nilotica* Willd. Ex. Del. also commonly called *Mimosa arabica* or *Mimosa nilotica*, is a tree which grows up to 20 m in height with a dense spheric crown; belonging to the subfamily Mimosoideae of the family Fabaceae [1]. *Acacia arabica* Willd. is widely distributed throughout the drier parts of India, Ceylon, Baluchistan, Waziristan, Arabia, Egypt, tropical Africa and Natal [2, 3]. Traditionally plant is used in cholera, hairfall, syphilis, gonorrhea, leucorrhoea, diarrhea, dysentery, diabetes, and is styptic, astringent, antiprotozoal, hypotensive, spasmolytic, hypoglycaemic, CNS depressant, anti fungal. Leaves are used for eye complaints, headache, throat infection, ulcers, antiscorbutic, urinary problems and gonorrhea [1-3]. Gum and bark is astringent, cooling, emollient, expectorant, constipating, liver tonic, aphrodisiac, haemostatic, antipyretic and tonic, useful in asthma, diarrhoea, dysentry, seminal weakness, haemorrages, leprosy, pharyngodynia, pneumonosis, haemorrhoids, urinogenital discharges, bums, colic, intermittent fevers and general debility, used to treat cancers and/or tumors (of ear, eye or testicles), indurations of liver & spleen, condylomas, and excess flesh [2-3]. Flowers are used for ear complaints & as tonic; stem for toothbrush and to treat smallpox; and roots in liver complaints, tuberculosis, aphrodisiac, impotence and to impart courage [1]. *A. arabica* Willd. plant is being screened for various biological activities and reported to have anti- free radical [4-6], anti-quorum sensing, chemopreventive [6-7], anthelmintic [8], antimutagenic [7,9], cytotoxic [9], antimicrobial [10, 11], anti-inflammatory [12], sexually transmitted infections [13]. *A. arabica* Willd. bark contains several polyphenolic compounds, (+)catechin, (-)epicatechin, (+)dicatechin, epigallo catechin, gallic acid & its methyl ester, quercetin, catechol, epicatechol, (+)-leucocyanidine gallate, sucrose and tannin [3,14,15]. Gum contains polysaccharides, calcium, magnesium, salts of arabic acid, malic acid, *D*-galactose, oxidative enzymes, *L*-rhamnose, *L*-arabinose and its derivatives along with four aldobiuronic acids viz. 6-*O*-(*β*-glucopyranosyluronic acid)-*D*-galactose, 6-*O*-(4-*O*-methyl-*β*-*D*-glucopyranosyluronic acid)-*D*-galactose, 4-*O*-(*α*-*D*-glucopyranosyluronic acid)-*D*-galactose and 4-*O*-(4-*O*-methyl-*α*-*D*-glucopyranosyluronic acid)-*D*-galactose. It also contains 3,5-di-*O*-methyl-*L*-arabinos, 2-*O*-*β*-*L*-arabinopyranosyl-*L*-arabinose, arabinobiose, 3-*O*-*β*-*L*-arabinopyranosyl-*L*-arabinose [14-16]. Seeds contains proteins, various amino acids, ascorbic acid, tannins and fatty acids [3]. Pods contains tannins [15], m-Digallic

acid, and chlorogenic acid, galloylated flavan-3,4-diol and 7,3',4',5'-tetrahydroxyflavan-3,4-diol[14]. Flower contains Kaempferol-3-glucoside, isoquercetin, leucocyanidin[3]; roots contain hentriacontane, n-hentriacontanol and paulowrin and heartwood yields betulin and sitosterol [14].

In the present study, established the necessary pharmacognostic standards and phytochemical constituents for evaluation of roots of *Acacia arabica* Willd. because roots are the essential part of plant and contains tannins and flavonoids which shows various therapeutic activity like antioxidants, hepatoprotective, wound healing, etc. Therefore, the present study was carried out to standardize the roots using chemical, botanical and analytical means so that this might be an important tool of identification for herbalists by using HPTLC.

MATERIALS AND METHODS

Plant material and extract preparation

Roots of *Acacia arabica* Willd. were collected from the surroundings of Sonepat. The plant material was identified by Dr. H.B. Singh (Scientist F and Head, Raw Materials Herbarium and Museum, NISCAIR, Delhi) under a voucher specimen number-NISCAIR/RHMD/Consult/-2009-10/1278/82 dated Oct. 1, 2009. The roots were cut into small pieces, then, subjected to shed drying and further crushed to coarsely powder. The shade dried and powered root was subjected to maceration with different solvents viz. petroleum ether (60-80°c), chloroform, ethyl acetate, ethanol (95%) and finally with water to get respective extracts. All extracts were individually filtered and evaporated to dryness. The dried extracts were weighed and percentage yields were determined respectively and stored in freeze condition for further use.

Pharmacognostical evaluation

Chemicals and instruments

Solvents viz. petroleum ether (60-80°c), chloroform, ethyl acetate, ethanol (95%), and reagents viz. phloroglucinol, glycerine, HCl, chloral hydrate and sodium hydroxide were procured from RFCL, Mumbai, India. Photographs of tissue arrangement were taken with Labomed ATC-2000 microscope attached with Sony camera. HPTLC was done using CAMAG HPTLC densitometer.

Macroscopic and microscopic analysis

The colour, shape, size, odour, fracture and surface texture of dried roots were observed. For microscopic study, thin hand sections were prepared and cleared with chloral hydrate; stained with phloroglucinol and hydrochloric acid, then mounted in glycerin for the identification of various regions. For powder study, powder (sieve no. 60) of dried root was taken, separately treated with phloroglucinol and hydrochloric acid, glycerin, iodine solution, ruthenium red solution, safranin solution [17].

Fluoroscence study

The powder material was treated separately with different reagents and exposed to visible and ultraviolet light. The Fluorescence nature of different extracts of roots was studied by using a minute quantity of petroleum ether, chloroform, ethyl acetate, ethanol and water extract. The extracts were put on the slide and observed under visible and UV light [18-21].

Physicochemical parameters

Physicochemical parameters adopted to confirm the purity and quality of drug. Total ash, water-soluble ash and acid-soluble ash were determined [17]. Ethanol-soluble, and water-soluble extractive values were determined [22]. Loss on drying and swelling index was also determined [17]. Preliminary phytochemical screening was carried out, by using standard methods, to identify the presence of various phytoconstituents [23].

Quantification of quercetin by HPTLC

A HPTLC densitometric method was developed for quantification of quercetin in the ethanolic extract of roots of *Acacia arabica* Willd. Sample was applied using CAMAG Linomat 5 "unknown" S/N 0.00 (00.00) instrument, with application parameters (Spray gas: Inert gas; Sample solvent type: Methanol; Dosage speed: 150 nl/s; Pre-dosage volume: 0.2 ul) & sequence (Syringe size: 100 µl; Number of tracks: 4; Application position Y: 10.0 mm; Band length : 8.0 mm).

Table 1: Sequence of application of sample & standard on TLC plate

Sl. No.	Appl. Position	Appl. Volume	Vial#	Sample ID	Active
1.	15.0 mm	5.0 µl	1	Sample A	Yes
2.	29.0 mm	10.0 µl	1	Sample A	Yes
3.	43.0 mm	5.0 µl	2	Std. Quercitin	Yes
4.	57.0 mm	10.0 µl	2	Std. Quercitin	Yes

Development of *TLC*-TLC was developed in glass tank (Twin Trough Chamber 10x10cm), pre-conditioning mobile phase [Toluene: Ethyl acetate: Methanol (4.4:5:0.6)] and dried at 60 °C using hair dryer for 5 Minutes.

Results and discussion

Pharmacognostic studies

Morphological studies

Acacia arabica Willd. roots was found reddish brown in colour, disagreeable in odour, fibrous and moderately hard to fracture, 5-35 inches length and 0.5-8 cm diameter in size, cylindrical in shape, fibrous in texture, rough in touch and also showed the presence of adventitious roots.

Fig.1: A: *Acacia arabica* Willd. Stem, leaves and fruit; B: *A. arabica* root

Microscopical studies

Transverse section of roots of *A. arabica* Willd. has a spherical transaction showed single layered epidermis, pith, xylem cells, medullary rays and vessels. Powder Microscopy of *A. arabica* Willd. showed the presence of cork cells & fibres.

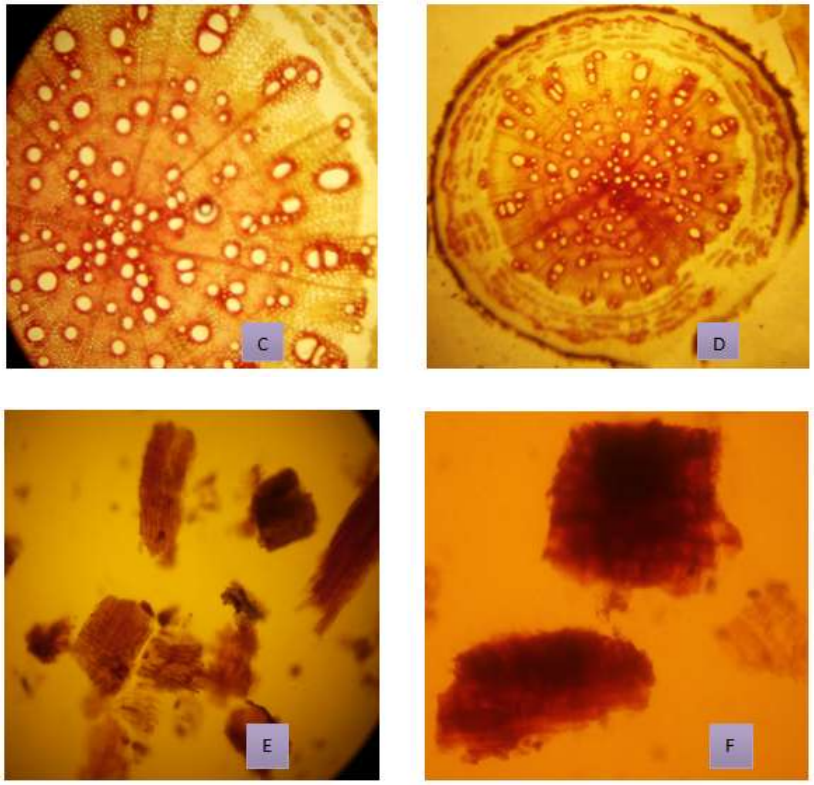

Fig. 2: C: Root microscopy (45X); D: Root microscopy (10X); E: Fibers (45X); F: Cork cells(100X)

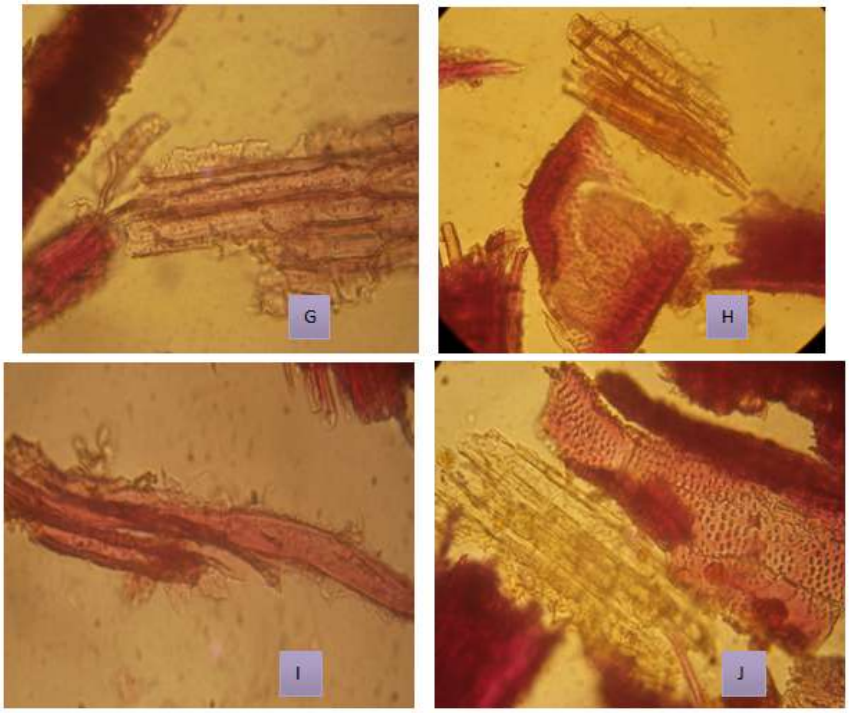

Fig. 3: G: Fibres and cork cells; H: Pitted cells, xylem and cells; I: Scleroid fibre with attaches Tracheid; J: Pitted fibres, xylem cells, phloem fibre and cork cells

Fluoroscence analysis

The Fluorescence nature of different extracts of roots was observed by using a minute quantity of petroleum ether, chloroform, ethyl acetate, ethanol and water extract under visible and UV light. The dried root powder treated with different chemical reagents viz. 1N NaOH in methanol, 1N NaOH in water, 1N HCl, 50% H_2SO_4, 50% HNO_3, 50% HCl and change in colour was observed under UV light.

Table 2: Fluorescence behavior of root powder of *A. arabica Willd.* with different reagents

Sl. No.	Treatment	Visible (400-800nm)	U.V. short (254 nm)	U.V. Long (366 nm)
1.	Powder as such	Reddish Brown	Light Brown	Brown
2.	Powder + 1N NaOH in Methanol	Yellowish Brown	Dark Green	Violet
3.	Powder + 1N NaOH in Water	Reddish Brown	Brown	Blackish Brown
4.	Powder + 1N HCl	Dark Brown	Brown	Violet
5.	Powder + 50% HNO_3	Yellowish Brown	Green	Dark Violet
6.	Powder + 50% HCl	Reddish Brown	Dark Green	Black
7.	Powder + 50% H_2SO_4	Dark Brown	Brownish Green	Dark Violet

Table 3: Fluorescence behavior of root extracts of A. arabica Willd. with different solvents

Sl. No.	Reagents	Colour of Extract			Extractive values
		Visible light	U.V. short	U.V. Long	
1.	Petrolium ether	Brown	Green	Violet	1.89%
2.	Chloroform	Dark Brown	Green	Dark Green	0.81%
3.	Ethyl acetate	Brown	Dark Green	Violet	0.85%
4.	Successive ethanol	Brown	Dark Green	Violet	5.21%
5.	Aqueous	Blackish Brown	Dark Green	Black	6.58%
6.	Total ethanolic	Dark Brown	Greenish Black	Violet	11.36%

Physicochemical constants parameters

Total ash 7.5% w/w, water-soluble ash 6.5% w/w, acid-insoluble ash 1.6% w/w, ethanol-soluble extractive value 12.0% w/w, water-soluble extractive values 6.9% w/w, loss on drying 12.0% w/w and swelling index Nil were calculated. Preliminary phytochemical screening of roots revealed the presence of flavonoids, tannins, saponin glycosides & reducing sugars.

Table 4: Phytochemical screening of *A. arabica* Willd. roots

Sl. No.	Compounds	Petrolium Ether	Chloroform	Ethyl Acetate	Ethanolic	Total Ethanolic	Aqueous
1.	Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve
2.	Carbohydrates	-ve	-ve	-ve	-ve	-ve	-ve
3.	Steroids	-ve	-ve	-ve	-ve	-ve	-ve
4.	Saponins	-ve	-ve	-ve	+ve	+ve	+ve
5.	Proteins	-ve	-ve	-ve	-ve	-ve	-ve
6.	Fixed Oils/Fats	+ve	-ve	-ve	-ve	-ve	-ve
7.	Flavanoids	-ve	-ve	+ve	+ve	+ve	+ve
8.	Tannins & Phenols	-ve	-ve	-ve	+ve	+ve	+ve
9.	Gums& Mucilages	-ve	-ve	-ve	-ve	-ve	-ve
10.	Glycosides	-ve	-ve	-ve	-ve	-ve	-ve
11.	Reducing Sugars	-ve	-ve	+ve	+ve	+ve	-ve
12.	Amino Acids	-ve	-ve	-ve	-ve	-ve	-ve

Quantification of Quercetin by HPTLC

Quantity of Quercetin in ethanolic extract of roots of *A. arabica* Willd. determined by HPTLC was 1.70%w/w.

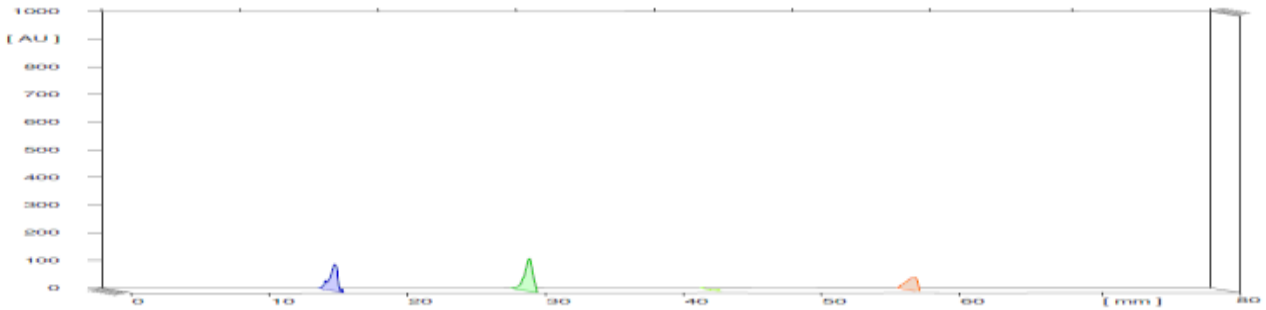

Fig. 3: HPTLC Peaks of *Acacia arabica* Willd. extract with Quercetin (All tracks at Wavelength)

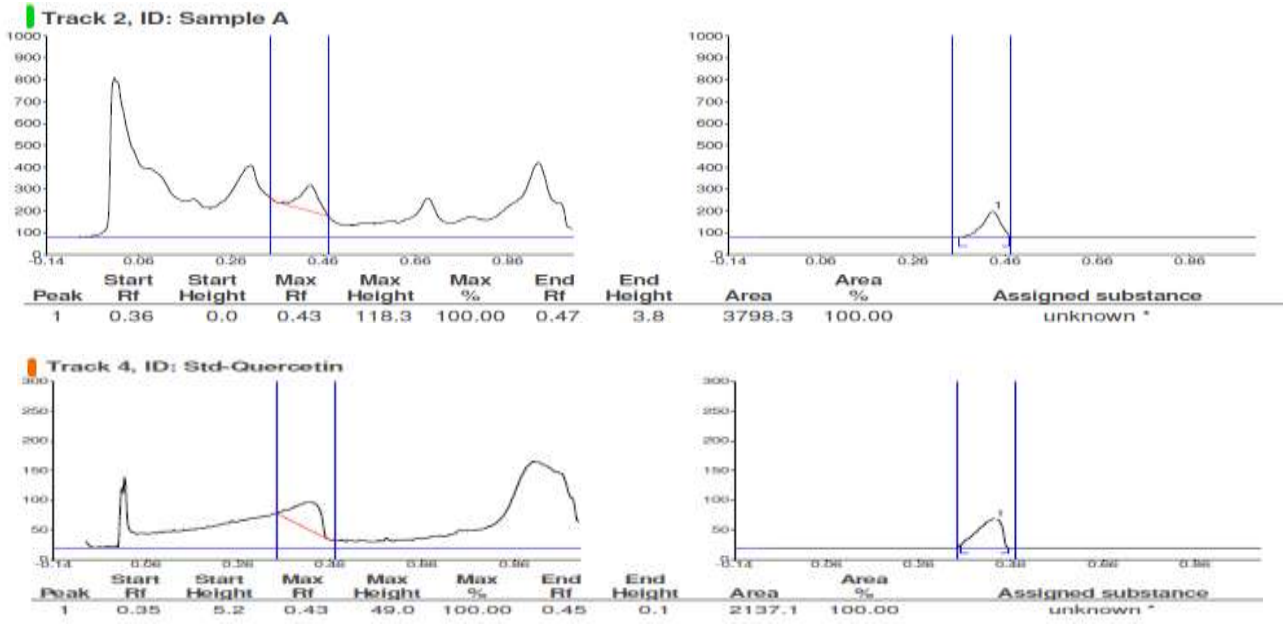

Fig. 4: Peaks of Quercetin Detected by HPTLC in Tracks of Sample and Standard

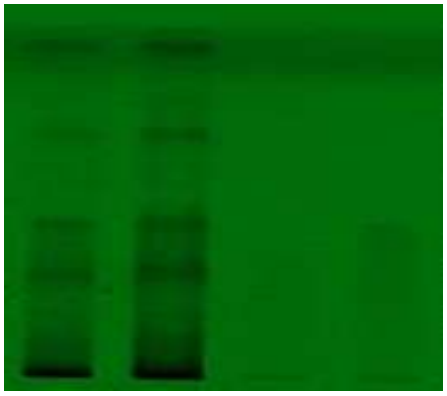

Fig. 5: HPTLC of Extracts with Standard at 366nm

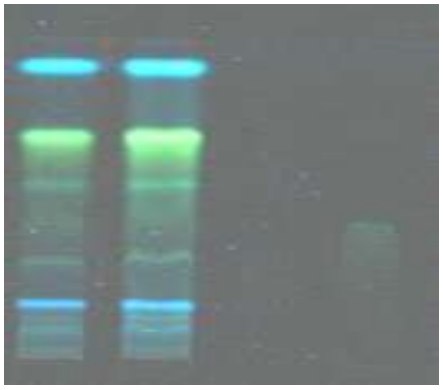

Fig. 6: HPTLC of Extracts with Standard at 254nm

CONCLUSION

From the present study, it was concluded that the plant contains tannins and flavonoids in its roots as main constituents and can be medicinally used for various purpose. As tannins and flavonoids further contribute to antioxidant activity, antiulcer and hepatoprotective activity; so these beneficial characters of the plant can be best utilized in the form of Medicament to treat such ailments. Moreover, there is a scope of manufacturing formulation either alone or in combination with other herbal extracts to prevent or treat various ailments.

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REFERENCES

1. *Acacia arabica*. Available from URL: http://en.wikipedia.org/wiki/Acacia_arabica
2. Kirtikar KR, Basu BD; Indian medicinal plants. Oriental Enterprises, Volume 4, 2003: 1285-1310.
3. Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in Ayurveda. New Delhi, Central council for Research in Ayurveda & Siddha, Dept. of ISM & Health Ministry of Health & Family Welfare (Govt. of India), 2002: 57-60, 216-219.
4. Singh R, Singh B, Singh S, Kumar N, Kumar S, Arora S; Anti-free radical activities of kaempferol isolated from *Acacia nilotica* (L.) Willd. Ex. Del. Toxicol in vitro, 2008; 22(8):1965-1970.
5. Sultana B, Anwar F, Przybylski R; Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees. Food Chem., 2007; 104(3):1106-1114.
6. Singh BN, Singh BR, Sharma BK, Singh HB; Potential chemoprevention of N-nitrosodiethylamine-induced hepatocarcinogenesis by polyphenolics from *Acacia nilotica* bark. Chem Biol Interact., 2009; 181(1): 20-28.
7. Kaur K, Arora S, Hawthorne ME, Kaur S, Kumar S *et al*.; A correlative study on antimutagenic and chemopreventive activity of *Acacia auriculiformis* A. Cunn. and *Acacia nilotica* (L.) Willd. Ex Del. Drug Chem Toxicol., 2002; 25(1): 39-64.
8. Bachaya HA, Iqbal Z, Khan MN, Sindhu ZU, Jabbar A; Anthelmintic activity of *Ziziphus nummularia* (bark) and *Acacia nilotica* (fruit) against trichostrongylid nematodes of sheep. J Ethnopharmacol., 2009; 123(2): 325-329.
9. Kaur K, Michael H, Arora S, Härkönen P, Kumar S; In vitro bioactivity-guided fractionation and characterization of polyphenolic inhibitory fractions from *Acacia nilotica* (L.) Willd. ex Del. J Ethnopharmacol., 2005; 99(3): 353-360.
10. Abd el Nabi OM, Reisinger EC, Reinthaler FF, Still F, Eibel U *et al*.; Antimicrobial activity of

Acacia nilotica (L.) Willd. ex Del. var. *nilotica* (Mimosaceae). J Ethnopharmacol., 1992; 37(1):77-79.
11. Saini ML, Saini R, Roy S, Kumar A; Comparative pharmacognostical and antimicrobial studies of *Acacia* species (Mimosaceae). J Med Plants Res., 2008; 2(12): 378-386.
12. Chaubal R, Mujumdar AM, Puranik VG, Deshpande VH, Deshpande NR; Isolation and X-ray study of an anti-inflammatory active androstene steroid from *Acacia nilotica*. Planta Med., 2003; 69(3): 287-288.
13. Kambizi L, Afolayan AJ; An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. J Ethnopharmacol., 2001; 77(1): 5-9.
14. Chatterjee A, Prakashi CS; The treatise of Indian of Indian Medicinal Plants. 1st edition, New Delhi, National Institute of Science Communication, Volume 2, Revised 2006: 48-53.
15. Nadkarni KM; The Indian Materia Medica. Bombay Popular Prakashan, Volume 1, 2000: 9-13.
16. The wealth of India- A Dictionary of Indian Raw Materials & Industrial Products. New Delhi, Publication and Information Directorate, CSIR, Volume 1-A, 1992: 23-47.
17. Trease, Evans; Pharmacognosy. International edition, W.B.Saunders, 2005: 15,456,481.
18. Indian Pharmacopoeia; Volume I, 4th edition, New Delhi, Ministry of health and family welfare, Govt. of India, 2007: 78
19. Chase CR, Pratt RS; Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Amer Pharm Assoc., 1949; 38: 324-331.
20. Ramakrishna V, Annie S, Gundu RP; Pharmacognostical studies of Eryngium foetidium Linn. Indian Drugs, 1991; 28(3): 124-130.
21. Venkatesh S, Swamy MM, Vijayalaksmi S, Reddy YSR, Suresh B; Pharmacognostical observations on Sida rhomboidea Roxb.- a report. Indian Drugs, 1994; 31(9): 421-425.
22. WHO. Quality control methods for medicinal plant material, Geneva. New Delhi, APTBS Publisher and Distributor, 1998: 10-30.
23. Khandelwal KR; Practical Pharmacognosy. 16th edition, Pune, Nirali Prakashan, 2006: 149-153.