WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 4, 629-637 Research Article ISSN 2277 – 7105

ISOLATION OF FLAVONOIDS FROM *BUTEA MONOSPERMA* (LAMARCK) KUNTZE.

Divya Fageria*, Renu Kumari and D. V. Rao

Laboratory of Bioactive Compounds and Biotechnology, Department of Botany, University of Rajasthan, Jaipur-302004, India.

Article Received on
29 Dec. 2018,

Revised on 19 Jan. 2018,
Accepted on 09 Feb. 2018,

DOI: 10.20959/wjpr20184-10929

***Corresponding Author**
Divya Fageria
Laboratory of Bioactive Compounds and Biotechnology, Department of Botany, University of Rajasthan, Jaipur-302004, India.

ABSTRACT

Plants has been reported as diverse reservoirs of secondary metabolites like tannins, flavonoids, sterols, alkaloids and triterpenes, etc., which has essential role in physiology, nutrition and treatment of many diseases. Flavonoids are major classes of phenolic compounds in angiosperms. The primary major function in this pattern is the screening of these phytochemicals in the plants. The chromatographic study of the compounds serves to be a very significant and dependable basis in the synthesis of natural products screening in plants. *Butea monosperma* is an significant medicinal flora having various therapeutic values like antioxidant, antimicrobial, and anticancer agents. It is an vital arid zone flora normally consumed as herbal drugs for treatment in pain and inflammation, along with other metabolic disorders such as diabetes and obesity. Hence in the present study, focus is to identify the flavonoid in different samples of *B. monosperma* by TLC and PTLC. Further, the separation of the similar products were confirmed by IR, HPLC and GC- MS analysis.

KEYWORDS: *Butea monosperma*; flavonoids; TLC; IR; GC-MS.

INTRODUCTION

Bioactive phytochemicals that present natively in floras are accountable for the color and chemical properties like deep purple color of blueberries and fragrance of garlic. These compounds are known as bioactive compounds. The word, phytoceuticals, is normally used to indicate to that chemical which possess therapeutic ventures. These are used in drugs since many decades. Most of secondary metabolites has been studied structurally for possible screening and their use in the harmful diseases like cancer. For first time current investigation

has been done in the identification and isolation of new bioactive components of medicinal importance from the experimental plant.[1-4]

Flavonoids are a phenolic substituent's synthesized by plants which bear similar structure. They can be easily identified as flower pigments in angiosperms. Though, their amount is not limited in flowers only, as they are present in every plant part. They are also known to play an important task in providing resistance to the plant species, like rotenone, which is a isoflavonoid, acting as an potent insecticide. The credible effect of these compounds on human health is also widely researched especially in the curing of cancer particular breast cancer.[5-8] Consumption of soy items rich in isoflavones has been imperceptibly connected with decrease in colon cancer.

Butea monosperma (palas) is a deciduous tree placed under family fabaceae-papilioneae. It is called 'Flame of the Forest' and Bastard Teak.[9] They belong to one important families of angiosperms, having 630 genera and 18000 species. It is cosmopolitan in Asian continent, mainly in Indo-gangetic plains.[10] It has been reported that this plant is treated as, 'God of Fire'. Flowers are delivered as a replacement of blood in sacrifice ceremony to goddess Kali.[11] The plant bears various medicinal uses like anticonvulsive,[12] hypoglycemic.[13]

MATERIALS AND METHODS

Extraction

Various plant samples (Leaves, flowers, Seeds and Roots) of *Butea monosperma* were collected from Amer, Delhi road Jaipur, India. The plant materials were taxonomically recognized and confirmed by Department of Botany, University of Rajasthan (RUBL 211650), Jaipur. The plant samples were cleaned, shade dried and powdered and kept for further use. These samples were finally rinsed with 80% methanol[14] for 24 h. Methanol soluble fractions were filtered, desolated *in vacuo* and were differentiated by sequential extraction with petroleum ether (Fr1), diethyl ether (FrII) and ethyl acetate (FrIII) each. Every method was done 3 times to isolate the compound completely, fraction 1 was discarded in each case as it had fatty substance, while fraction II and III were resoluted and used for identifying flavonoids.

Fraction III was more reacted by adding with 7% H_2SO_4, filtered and filtrate was isolated three times with ethyl acetate. All ethyl acetate layers were pooled out and pH was

maintained by distilled water and dried *in vacuo*. Both fractions II and III were diluted in ethanol (2-5mL) for TLC.

Qualitative

Thin Layer Chromatography (TLC)

Glass plates of size (20x20 cm) were encrusted with Silica gel G (250mμ thick). The new plates were evaporated at room temperature; and further heated at 100 ^{0} C for 30 min to activate and collected for further analysis. Every sample was co- chromatographed with standards (quercetin, luteolin, kaempferol). These plates were saturated with different solvent systems (Benzene: Acetic Acid: Water::125:72:3.[14] The developed plates were evaporated and seen under UV light with little exposure to ammonia fumes. The developed plates were sprayed with 5% $FeCl_3$, 0.1% alcoholic $AlCl_3$ and fumigated with I_2 chamber. The coloured spots developed were recorded and the Rf value of each spot was noted down. Other solvent mixtures having tertiary butanol, acetic acid, water (3:1:1), n- butanol, acetic acid, water (4:1:5), were also experimented, but solvent system of benzene, acetic acid, water (125:72:3) was found to be best among all the tested solvents.

Preparative thin layer chromatography (PTLC)

PTLC of above extracts were coated with silica gel G having 500mμ thickness by marking the extract along with standards. These plates were saturated in the solvent mixture of benzene, acetic acid, and water (125:72:3), evaporated and visualized under UV light. Each of spots resembling with the standard were noted, eluted from 200 plates, and isolated with 50% CH_3OH. The separated fractions were filtered, evaporated and again spotted along with standard to confirm their purity which were subjected to crystallization separately and UV (Ultraviolet and visible spectrophotometer; Carl Zeiss, Jena, DDR, VSU-2P spectrophotometer) and IR (Perkin, Elmer 337,Grating Infra-red spectrophotometer), melting point ,mixed melting point(Toshniwal Melting Point Apparatus)was estimsated.

Quantification

The isolated flavonoids were calculated by spectrophotometer using established protocol.[14]

Stock solution ($1mgL^{-1}$) of all standards were prepared by dissolving markers in methanol. Different stocks from 20μg to 160μg of every samples were marked separately on silica gel G plates. These developed plates were evaporated andseen under UV light. The fluorescent spots were noticed and separated along with the absorbance in different test tubes. Methanol

of IR grade (5mL) was added to each test tube, stunnedro bustly, centrifuged and supernatants were eluted separately. Every sample was rinsed to 10mL by increasing the concentration of methanol. To each sample, 3mL of 0.1 M $AlCl_3$ solution was added again stunned robustly and kept at room temperature for 20 min. 5 replicates done in each case and their O.D. were measured at 426nm for kaempferol and luteolin and at 440nm for quercetin against blank (10ml of \methanol and 3mL of 0.1 M $AlCl_3$). The standard curves were drawn between concentration and their respective average O.D. of each of the compound.

Every extract (fr. II and III) was dissolved in 5 mL of methanol and 0.1mL was marked on silica gel G marking with standard separately. The plates were developed as above and the spots resembling with that of standard were marked on each plate under UV. Each spot was isolated with the silica gel, in methanol and plant extracts were prepared in the same way as described above. The O.D. in each case was recorded and concentration of each sample was drawn usingthe regression curve of standards flavonoids. The concentrations were quantified on mg/g dry weight basis.

GC-MS analysis

Gas Chromatography and Mass Spectroscopy (GC-MS) The extract and the standard samples were analyzed by GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with using Agilent 7890A/5975C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m×0.25 mm i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100 °C (3 min) to 280 °C at 1 to 40 °C/min; detector temperature, 250 to 280°C; carrier gas, He (0.9 mL/min). Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of the Wiley library or with the published mass spectra

RESULTS

Three spots of flavonoids were observed in different plant parts of *Butea monosperma*on TLC plates developed and sprayed with 5% $FeCl_3$.The R_f values of these spots matched with their respective authentic standards and were identified as kaempferol, quercetin, luteolin. Solvent system Benzene: Acetic Acid: Water (125:72:3) gave best results with R_f values viz., kaempferol, 0.86; luteolin, 0.56; quercetin, 0.78 **(Table-1).** When other solvents viz n-Butanol: Acetic acid: Water (4:1:5) and conc. HCl: Acetic acid : Water (3:30:10) the $R_f$value

of kaempferol was found to be 0.83 and 0.55, that of quercetin was found to be 0.64 and 0.41 while that of luteolin was found to be 0.83 and 0.77, respectively.

Table 1: Chromatographic behavior and physicochemical characteristics of isolated flavonoids identified in different plant parts of *Butea monosperma.*

Isolated compounds	R_f value			Physical appearance			Color after spray				Melting point (°C)	IR Spectral Peaks ν (KBr) cm^{-1}
	S_1	S_2	S_3	Day light	UV ammonia	Iodine Vapor	R_1		R_2			
							Visible	UV	Visible	UV		
Kaempferol	0.86	0.83	0.55	GN-YW	BT-YW	YW-BN	BN	BK	YW	YW-GN	276-278	(O-H) (3410cm-1(270, 295, 344 , 1690
Luteolin	0.56	0.83	0.77	GN-YW	YW	YW-BN	TN	BK	DL-YW	YW-GN	326-329	3421, 2965, 1736 (lactone), 1510 (furan), 1461, 1388, 1360, 1274, 1242, 1187, 1136, 1028, 903, 850 cm^{-1}.
Quercetin	0.78	0.64	0.41	GN-YW	YW	YW-BN	BT-GY	BK	DL-YW	YW-GN	315-320	3423, 1739, 1655 (O-H), 1608, 1508, 1305, 1203 (C=C), 1088.

Abbreviations:

S_1 – Benzene : acetic acid : water (125 : 72 : 3), S_2 – n-Butanol : acetic acid : water (4 : 1 : 5), S_3 – Conc. Hydrochloric acid : acetic acid : water (3 : 30 : 10), R_1 – 5% $FeCl_3$ solution, R_2 – 5% alc. $AlCl_3$ solution, YW – Yellow, BK – Black, BN – Brown, BT – Bright, DL – Dull, GN – Green, GY – Gray.

The isolated flavonoids viz., kaempferol, quercetin, luteolin were also identified and characterized by super imposable IR peaks **(Fig. 1,2,3.),** mp (kaempferol, 271-273 °C; luteolin327-328 °C; quercetin 309-311°C and UV maxima (nm) in methanol (kaempferol, 253sh, 266, 394sh, 322sh, 368; quercetin 255, 269sh, 301sh 374;luteolin 242sh, 253, 267, 291sh, 349 which were similar to the respective authentic standards.

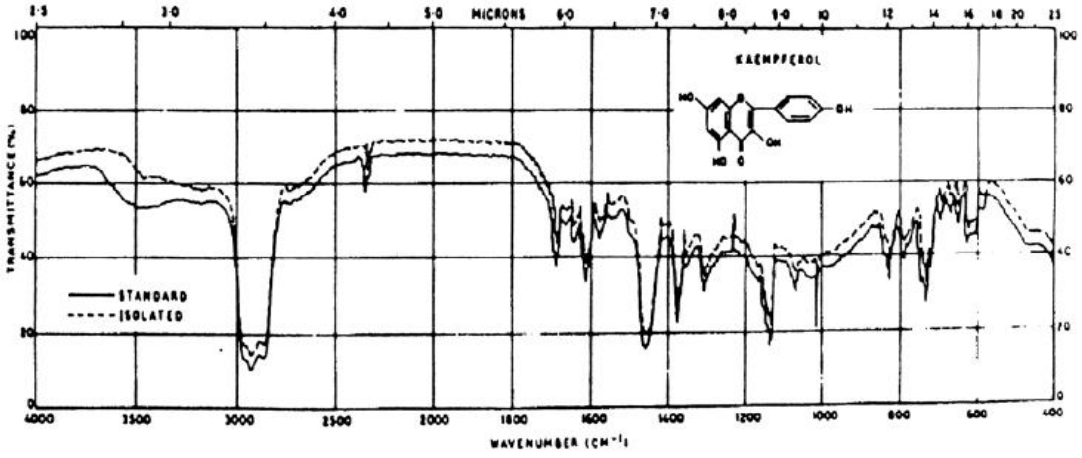

Fig. 1: IR Spectra of kaempferol.

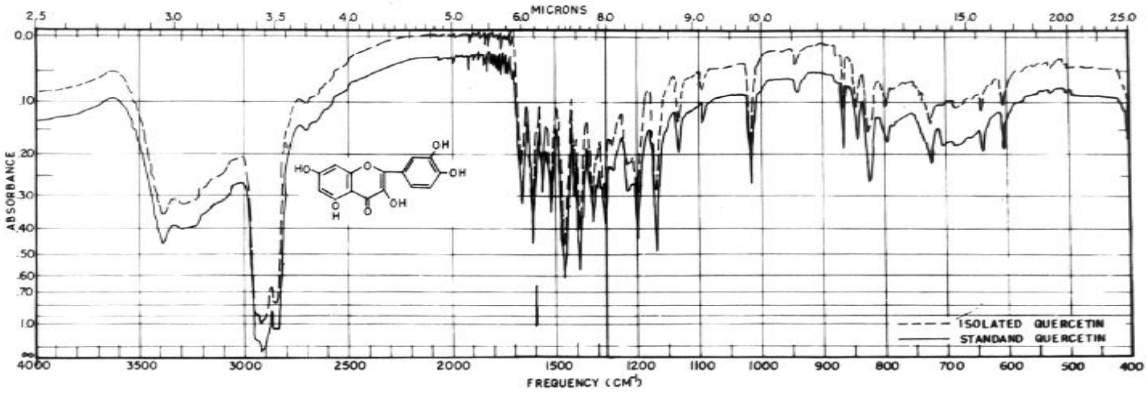

Fig. 2: IR Spectra of Quercetin.

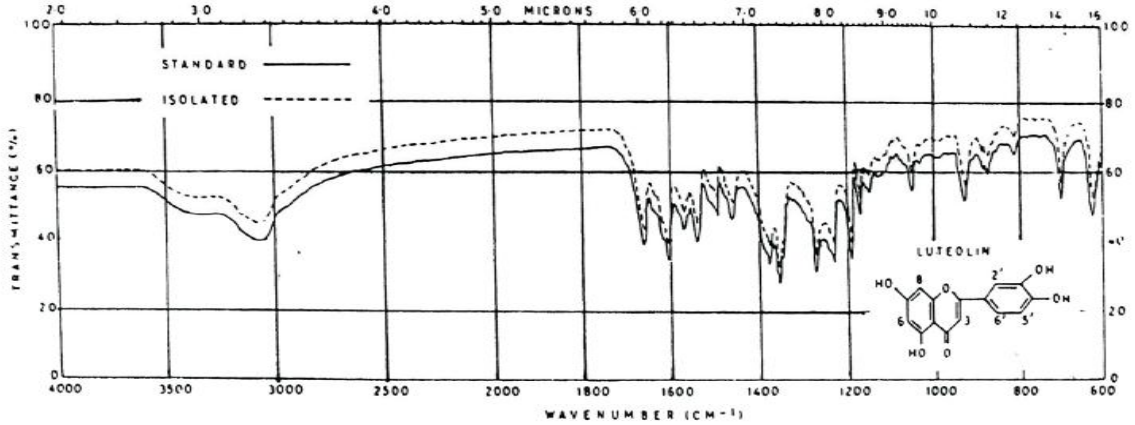

Fig. 3: IR spectra of luteolin.

Quantitative analysis

Among the plant parts total flavonoid content (free & bound) was maximum in seeds (1.21mg/gdw), and minimum (0.55mg/gdw) in Roots. Flavonoid content in its free form was more as compared to the bound form in all plant parts. Individually, all the isolated flavonoids were more in seedswith the maximum level of quercetin (0.36mg/gdw) followed by kaempferol and luteolin (0.22mg/gdw and 0.12mg/gdw) **(Table 2).**

Table 2: Flavonoids content (mg/gdw) in different plant parts of *Butea monosperma*.

S. No.	Plats Parts	Free flavonoids (mg/gdw)				Bound flavonoids (mg/gdw)				Total flavonoids (free+bound) (mg/gdw)
		K	Q	L	T	K	Q	L	T	
1.	Flowers	0.19	0.34	0.10	0.63	0.15	0.18	0.07	0.40	**1.03**
2.	Seeds	0.22	0.36	0.12	0.70	0.18	0.24	0.09	0.51	**1.21**
3.	Leaves	0.13	0.20	0.10	0.43	0.10	0.16	0.05	0.31	**0.74**
4.	Roots	0.10	0.14	0.09	0.33	0.08	0.12	0.02	0.22	**0.55**

GC-MS analysis

When flavonoid extract of flowers of *B. monosperma*were analysed by GC MS about 46 compounds were observed. Maximum retention time (28.27 min) was observed in 2Heptadecenal. Maximum area was observed in n Hexadecanoic acid (43.01%) (**Table 3 and Fig. 4).**

Table 3: GC MS analysis of flavonoids from *Butea monosperma*.

RT	Compound Name	Area	Area %
4.12	Dimethylsulfoxonium formylmethylide	11112148	0.11
5.12	Furan, 2pentyl	7447023	0.08
5.52	Propanedioic acid, propyl	10322499	0.10
7.10	Nonanal	12400868	0.12
11.59	2,4Decadien1ol	17730830	0.18
12.66	Cyclohexanemethyl propanoate	7389641	0.07
13.99	1Hexyl2nitrocyclohexane	23864212	0.24
14.36	2HPyran, 2(tertbutylthio) tetrahydro	10719718	0.11
15.50	5Ethyl2(furfurylidenehydrazino)2thiazolin4one	21748899	0.22
16.02	1,2,4,5Tetroxane, 3,3,6,6tetraphenyl	6303800	0.06
16.38	2Propenoic acid, tridecyl ester	7595347	0.08
16.63	Azelaic acid	88922928	0.90
17.55	2Hexyl1octanol	14630609	0.15
18.14	Cyclohexanemethyl propanoate	11092058	0.11
18.78	Oxalic acid, allyl hexadecyl ester	7548409	0.08
18.95	Ditertbutyl carbonotrithioate	8614599	0.09
19.33	Pentadecanoic acid, 14methyl, methyl ester	41472482	0.42
20.19	nHexadecanoic acid	4267927471	43.01
20.54	2,6,10,14Tetramethylpentadecan6ol	8725289	0.09
20.54	9,9Dimethoxybicyclo[3.3.1]nona2,4dione	14612220	0.15
21.15	2Hexyl1octanol	16743927	0.17
21.15	2Hexyl1octanol	8335970	0.08
21.30	Benzoic acid, 3(5hydroxy1pentenyl), methyl ester, (E)	7114976	0.07
21.39	trans13Octadecenoic acid, methyl ester	38213475	0.39
21.63	Hexadecanoic acid, 15methyl, methyl ester	27695181	0.28
22.16	cisVaccenic acid	2761328159	27.83
22.35	Octadecanoic acid	1041154079	10.49
22.59	Z,Z10,12Hexadecadien1ol acetate	182649489	1.84
22.85	(R)() 14Methyl8hexadecyn1ol	13437397	0.14
22.99	Z,Z10,12Hexadecadien1ol acetate	168894850	1.70
23.35	2Hexyl1octanol	7267553	0.07
23.66	1,5Diphenylhex3ene	17611001	0.18
23.73	Tetradecanoic acid, 10,13dimethyl, methyl ester	12491516	0.13
23.89	Cyclohexane, 1(1,5dimethylhexyl) 4(4methylpentyl)	7033580	0.07
23.99	Z4Dodecenol	7110652	0.07
24.10	cis11Eicosenoic acid	68114421	0.69
24.21	1Hexyl2nitrocyclohexane	15734213	0.16
24.31	Eicosanoic acid	197866702	1.99

24.47	4Tetradecanol	8473499	0.09
24.86	1Hexyl2nitrocyclohexane	41044140	0.41
25.00	2,7Octadiene1,6diol, 2,6dimethyl, (Z)	14646735	0.15
25.28	Sulfurous acid, butyl tetradecyl ester	9721576	0.10
25.52	2Hexyl1octanol	7627186	0.08
25.67	Hexadecanoic acid, 15methyl, methyl ester	45346965	0.46
26.11	2Hexyl1octanol	6760610	0.07
26.25	Octadecanoic acid	515654946	5.20
27.46	Tetradecanoic acid, 10,13dimethyl, methyl ester	16112556	0.16
27.85	Cyclohexane, 1(1,5dimethylhexyl) 4(4methylpentyl	5472110	0.06
28.05	Eicosanoic acid	44522696	0.45
28.27	2Heptadecenal	9304161	0.09

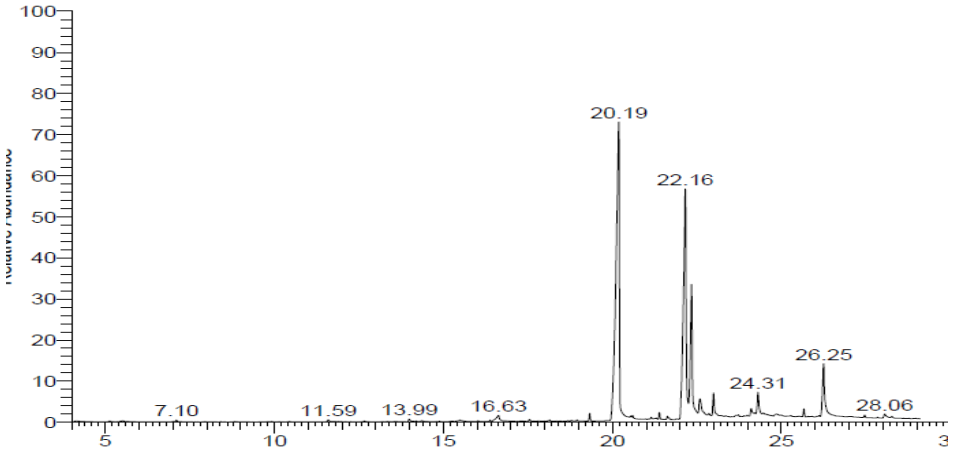

Fig. 4: GC MS analysis of flavonoids from *Butea monosperma.*

DISCUSSION

Flavonoids are natural product phenolic glycosides synthesized from aromatic amino acids, occur almost naturally in angiosperms. They provide colour to flowers and fruits, which has major task in appeal of pollinating insects. Flavonoids have also been reported to have pathological significance in plants by providing resistance to the plants against pests and insects[15] besides physiological importance for animals. The flavonoids generally occurs in their free form and at reactive sites in their bound form as glycosides. Therefore, the variation in amount between free and bound forms shows their role at resting and active stages, thus giving higher or lower recovery of free and/or bound flavonoids.[16]

CONCLUSION

The present investigation has been done to isolate and identify flavonoids found in *Butea monosperma* using IR, and GC-MS. The occurrence of these natural products in *Butea monosperma* lends credence to its use for welfare of mankind. It also accounts for the production of novel medicines with isolation of specific compounds.

ACKNOWLEDGMENT

The authors are thankful for Head, Department of Botany, and University of Rajasthan for giving necessary facilities.

REFERENCES

1. Khan, M R, W Rizvi, G N Khan, R A Khan, and S Sheen. "Carbon tetrachloride induced nephrotoxicity in rats: Protective role of Digeramuricata." *J. Ethnopharmacol.* 122 (2009): 91-99.
2. Khan, R A, M R Khan, and S Sahreen. "Evaluation of Launaeaprocumbens use in renal disorders: A rat model." *J. Ethnopharmacol.* 128 (2010a): 452-461.
3. Khan, R A, M R Khan, S Sahreen, and J Bukhari. "Prevention of CCl4 induced nephrotoxicity with Sonchusasper in rat." *Food Chem. Toxicol.*23 (2010b): 1304-1321.
4. Sahreen, S, M R Khan, and R A Khan. "Evaluaion of antioxidant activities of various solvent extracts of Carissa opaca fruits." *Food Chem.* 122 (2010): 1205-1211.
5. Cornwell, T, W Cohick, and I Raskin. "Dietary phytoestrogens and health."*Phytochemistry*, no. 65 (2004): 995-1016.
6. Dixon, R A. "Phytoestrogens." *Annu. Rev. Plant Biol.* 55 (2004): 225-261.
7. Greenwald, P. "Clinical trials in cancer prevention: Current results and perspectives for the future." *J. Nutr.* 134, no. 12 (2004): 3507-3512.
8. Holzbeierlein, J M, McIntosh, and J B Thrasher. "The role of soy phytoestrogens in prostate cancer."*Curr.Opin. Urol.* 15 (2005): 17-22.
9. Kirtikar KR, Basu BD (1995). Indian Medicinal Plants. Vol.1, International book distributors, Dehardun, India, pp.830-832.
10. Chopra, R.N.,Chopra, J.C., Handa, K.L. and Kapur,L.D., Indigenous drugs of India, 1958.
11. Ambasta, B.P., 1994. The useful plants of India. CSIR, New Delhi 1994; 1-91.
12. Kasture,V.S., Chopde C.T. and Deshmukh V.K. Anticonvulsive activity of Albizzia lebbeck, Hibiscus rosasinesis and Butea monosperma in experimental animals. J of Ethnopharmacology, 71 (2000): 65–75.
13. Somani, R. Kasture, S. and Singhai, A., Antidiabetic potential of Butea monospermain Rats, Fitoterapia, 77 (2006): 86-90.
14. Subramanian SS and Nagarajan S. 1969. Flavonoids of seeds of *Crotalaria retusa*and *C. striata*.Curr. Sci., 38: 365.
15. Harborne JB Mabry TJ and Mabry H. (Eds.). 1975. The Flavonoids. Chapman & Hall. London, UK.