International Journal of Chemical Studies

P-ISSN 2349–8528
E-ISSN 2321–4902
IJCS 2016; 4(6): 78-82

Received: 11-09-2016
Accepted: 12-10-2016

Florence AR
Department of Botany, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu, India

Regini Balasingh GS
Department of Botany, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu, India

Correspondence
Florence AR
Department of Botany, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu, India

Phytochemical analysis of *Gmelina asiatica* L. leaves

Florence AR and Regini Balasingh GS

Abstract
Objective: The present study was undertaken to investigate the bioactive components present in the leaf extracts of *Gmelina asiatica*.
Methods: Aqueous, petroleum ether, chloroform, ethanol and acetone extracts were prepared by adding 100 g of leaf powder to 1000 ml of these solvents and subjected to soxhlet extraction. The extracts were concentrated by using vacuum evaporator and dried at 60 °C. Preliminary phytochemical screening was performed by Harborne method. Total tannin and flavonoid content was determined by using Folin Ciocalteau reagent and Aluminium Chloride method respectively.
Results: Different extracts showed the bioactive components such as alkaloids, carbohydrates, glycosides, coumarins, quinones, saponins, steroids, terpenoids, proteins, phytosterols, tannins and flavonoids. Total tannins content in the ethanolic leaf extract was estimated as 0.042µg/µl and flavonoids content was estimated as 0.045µg/µl.
Conclusion: The findings of the study revealed that various chemical constituents present in the leaf extracts of *G. asiatica* is rich in phytopharmaceutical importance.

Keywords: *Gmelina asiatica* L., leaf extract, phytoconstituents, solvents, Verbenaceae

1. Introduction
In developing countries medicinal plants and their components produce a diverse assortment of secondary metabolites of therapeutic importance and widely used in human therapy, veterinary, agriculture, scientific research and other countless areas [1-4]. Generally plants are the potential and inexhaustible sources of secondary metabolites which are able to synthesize a variety of chemical substances such as proteins, aminoacids, alkaloids, terpenes, flavonoids, glycosides, resins, saponins, volatile oils, gums, tannins, etc. which are responsible for medicinal activity [5-8]. Therefore, the most of the ongoing search for biologically active secondary metabolites and dietary supplement derived from plants have increased [9].
Among the secondary metabolites flavonoids and tannins are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants [10]. Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity [11]. Flavonoids from medicinal plants are safe and bioactive, dietary flavonoids are recognized for their antioxidant [12,13], anti-proliferative [14], antimicrobial [15-18], anti-allergic, anti-inflammatory, antihepatotoxic, antiulcer, antiviral and antispasmolytic effects [19-22] which may protect the body from various diseases and anticancer activities [23]. Tannins exhibit diverse biological activities such as antidysenteric, antidiarrheal, antimicrobial, antioxidant [24, 25], anthelmintic, antiviral [26], cytotoxic and antineoplastic agents [27].
Gmelina asiatica L. (Syn: *Gmelina parvifolia* Roxb.), is a deciduous large sized bush or shrub belonging to the family Verbenaceae which comprises about 35 species and 2 subspecies spread over in tropical and temperate regions of Asia. It is commonly called “Asiatic Bush Beech” and “Nilakumizh” in Tamil. The whole plant is medicinally important and well documented as a source of bioactive components with medicinal properties such as antimicrobial [28-32], anti-inflammatory [31], antioxidant [33, 34], antihyperglycemic and hypoglycemic [35], hepatoprotective [33], antipyretic [36], nematicidal [37] and anticancer activity [38-40]. The aerial parts and roots are used in traditional medicine for the treatment of jaundice, rheumatism, syphilis, gonorrhea, burning sensation of eyes, fever, dysuria, wounds, dandruff, diabetes, hepatic diseases, catarrh of the bladder, blood purifier and also to reduce body heat [29, 30, 41-44]. The present study provides valuable information about secondary metabolites present in *Gmelina asiatica* leaf extracts.

2. Materials and Methods

2.1 Collection and extraction of plant materials

Fresh leaves of *Gmelina asiatica* were collected from Scott Christian College Campus, Nagercoil, Kanyakumari District, South Tamil Nadu, India and identified by using taxonomic keys [45]. The collected leaves were shade dried at room temperature for about two weeks to dry. They were made into powder with the help of a mechanical grinder and sieved. The powdered leaves were used for extraction. The dried powdered sample (100 g) of *G. asiatica* was extracted with 1000 mL of solvents such as aqueous, petroleum ether, chloroform, ethanol by a Soxhlet apparatus separately. The resultant filtrate was concentrated in powdered form by evaporation of the solvents using Rotary evaporator which was stored in a refrigerator at 4 ºC used for phytochemical analysis as per the standard methods of Harborne [46].

2.2 Estimation of Flavonoids

Total flavonoid content in the extract was determined by the aluminium chloride calorimetric method [47] using gallic acid as the standard; 1mL of test sample and 4 mL of water were added to a volumetric flask (10 mL volume). After 5 min, 0.3 mL of 5% sodium nitrite and 0.3 mL of 10% aluminium chloride were added. After 6 min of incubation at room temperature, 2 mL of 1 M sodium hydroxide (NaOH) was added to the reaction mixture. Immediately the final volume was made up to 10 mL with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as gallic acid equivalents in mg gallic acid/g dried extract.

2.3 Estimation of Tannins

Tannin content of the leaf extract was estimated by Folin Ciocalteau spectrophotometrically method [48]. One millilitre of the extract was mixed with 5 mL of vanillin hydrochloride reagent (a mixture of equal volumes of 8% HCl in methanol and 4% vanillin in methanol). The mixture was allowed to stand for 20 min and the absorbance measured at 500 nm against reagent blank. The standard graph was plotted for working standard catechin solution (0 to 250 µg/µL).

3. Results

The qualitative phytochemical analysis of aqueous, petroleum ether, chloroform, ethanol and acetone extracts of *G. asiatica* leaf revealed the presence of alkaloids, carbohydrates, glycosides, coumarins, quinones, saponins, steroids, terpenoids, proteins, phytosterols, tannins and flavonoids. Aqueous extract showed the presence of carbohydrates, flavonoids, tannins, terpenoids, steroids, phytosterols and alkaloids. The petroleum ether extract showed the presence of glycosides, quinones, coumarins and phytosterols. Chloroform extract revealed the presence of carbohydrates, glycosides, proteins, coumarins and phytosterols. The ethanol extract showed the presence of glycosides, flavonoids, tannins, saponins, terpenoids, steroids, proteins, coumarins and phytosterols. The acetone extract revealed the presence of carbohydrates, glycosides, tannins, saponins, proteins, coumarins and phytosterols. The maximum phytoconstituents present in ethanolic extract showed the presence of maximum number of (9/12) compounds and the minimum phytoconstituents were noticed in petroleum ether extract (Table 1; Fig. 1).

Table 1: Preliminary Phytochemical analysis of different extracts of *Gmelina asiatica* L. leaf

Phytoconstituents	Leaf extracts				
	Aqueous	Pet. ether	Chloroform	Ethanol	Acetone
Carbohydrates	+	-	+++	-	+
Glycosides	-	+	+++	+++	+
Flavonoids	+++	-	-	+	-
Tannins	++	-	-	+	+++
Saponins	-	-	-	+	+
Terpenoids	++	-	-	+	-
Steroids	+++	-	-	+++	-
Proteins	-	-	+	++	+++
Quinones	-	+	-	-	-
Coumarins	-	++	+	+++	+
Phytosterols	+	+	++	+	+
Alkaloids	+++	-	-	-	-

Abbreviation: (-) Absent; (+) Low; (++) Average; (+++) High

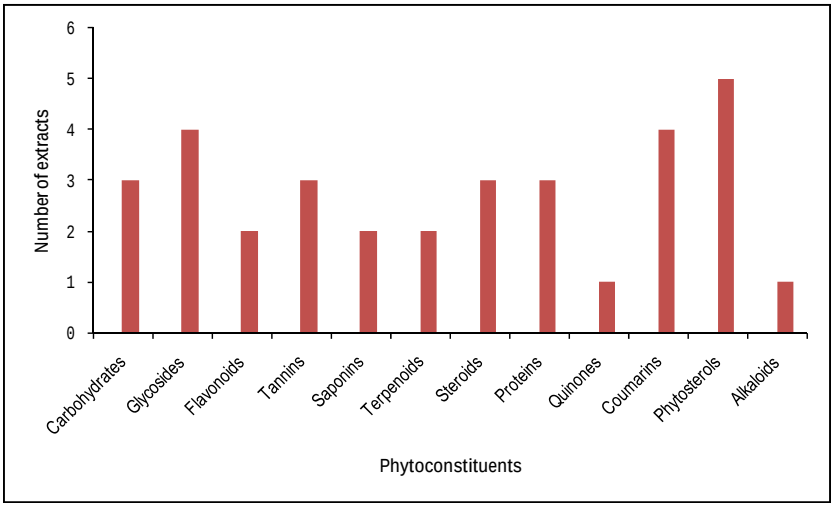

Fig. 1: Preliminary phytochemical screening of *Gmelina asiatica* L. leaf extracts

The quantitative phytochemical analysis of total tannin and flavonoid content of *G. asiatica* leaves in ethanol extract was studied. The total tannin and flavonoid contents are expressed as µg catechin equivalents/µl of extract and µg gallic acid equivalents/µl of dry weight of the extract respectively. Tannins content in the ethanolic leaf extract was estimated as 0.042µg/µl and flavonoids content was estimated as 0.045µg/µl.

4. Discussion

Plants are the potential sources of medicinal compounds in human life, as the major source of food, maintenance and improvement of health by elimination of the diseases causing microbes [49, 51]. Various medicinal plants have been used for years in daily life to treat diseases all over the world. Over 50% of all modern clinical drugs are herbal in origin and natural products play an important role in drug development programs [52]. With the development of pharmaceutical industries, much more interest has been created on plants and their products [53, 54].

Qualitative phytochemical analysis of aqueous, petroleum ether, chloroform, ethanol and acetone extracts of *G. asiatica* leaf indicated the presence of alkaloids, carbohydrates, glycosides, coumarins, quinones, saponins, steroids, terpenoids, proteins, phytosterols, tannins and flavonoids. Among the tested extracts, the ethanolic extract showed the presence of maximum number of (9/12) compounds. This is because ethanol is much polar than chloroform and acetone, hence extracting many of the active ingredients from the plant parts [55]. Several workers indicated that maximum separation of phytochemicals in the ethanolic extract than other solvents [31, 34,]. The above result indicates that the plant showed more amount of glycosides in all extracts but absent only in aqueous extract. Observations by Savithramma *et al*. [8] and Rajesh *et al*. [56] confirmed the absence of glycosides in aqueous extracts which is also proved by the present study. The presence of glycosides indicates that *G. asiatica* may be a potent in curing cardiac insufficiency, cough and circulatory problems and may acts as good sedatives and have antispasmodic properties [57], whereas alkaloids were absent in all extracts except aqueous extract. Previous works also supported the absence of alkaloids in *G. asiatica* leaf [29, 31, 58] but another work reported the presence of alkaloids in aqueous extract of *G. asiatica* leaf [29, 8]. Phytosterols were found in all the extracts studied. Phytosterols indirectly and directly inhibit the growth and metastasis of prostate cancer (PC-3) cells [59]. The earlier findings in different parts of *G. asiatica* extracts were reported in leaf [29, 31,]. When compared to root and stem, leaf extracts contributed wide range of secondary metabolites, because nutrients are abundant in leaves during photosynthesis. It is evident from the present study that the leaf of *G. asiatica* produces different types of secondary metabolites.

In quantitative phytochemical analysis, the total tannins and flavonoids in the ethanolic extract of *G. asiatica* leaves was 0.042 and 0.045µg/µl respectively. According to Salminen *et al*. [60] several environmental factors may be related to the low tannin content of the present study, such as season, area of collection site, effect of air pollution, nutrient restriction of soil and also depend on the type of solvent used, its concentration, the reaction time, temperature and concentration of catechin. In addition, the presence of phenolics and flavonoids are depended on plant parts, maturity at harvest, growing conditions, soil conditions and post-harvest treatment [61, 63]. Accurate estimation of flavonoids is difficult, because of the wide varieties of flavonoids available and the extensive distribution in various plants [64]. The estimated amount of total tannins and flavonoids from *G. asiatica* leaves in the present study is lower than those reported previously from *G. asiatica* stem [34]. The quantification of total tannins and flavonoids in the methanolic extract of the present study is more or less similar with *Verbena tenara*, *V. venosa* and *V. rigda* [65]. Earlier studies have confirmed the amount and composition of phenols and flavonoid compounds diversified at the sub-cellular level and within plant tissues as well [66, 67].

5. Conclusion

From the finding of this research, the plant *G. asiatica* leaf under investigation showed various phytoconstituents in the tested extract and their medicinal potential can be used as valuable drugs with antimicrobial, antioxidant, antidiabetic, anti-inflammatory and anticancer properties which also proves its effectiveness in curing various diseases.

6. References

1. Croteau R, Toni M, Norman K, Lewis G. Natural products (Secondary metabolites). In: Biochemistry and Molecular Biology of Plants Eds., Buchanan W, Gruissem R Jones, American Society of Plant Physiologists, 2000, 1250-1318.
2. Terryn N, Mantagu V, Inze D, Goossens A. Functional genomic approaches to study and engineer secondary metabolism in plant cell cultures. In: Medicinal and aromatic plants. Eds., Bogers RJ, Craker LE, Lange D, Springer Verlag, Netherlands, 2006, 291-300.
3. Vasu K, Goud JV, Suryam A, Charya MAS. Biomolecular and phytochemical analysis of three aquatic angiosperms. African Journal of Microbiology Research. 2009; 3:418-421.
4. Bobbarala V, Bramhachari PV, Ravichand J, Reddy K, Kotresha D, Chaitanya KV. Evaluation of hydroxyl radical scavenging activity and HPTLC fingerprint profiling of *Aegle marmelos* (L.) Correa extracts. Journal of Pharmacy Research. 2011; 4(1):252-255.
5. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian Journal of Pharmacology. 2000; 32:S81-S118.
6. Zwenger S, Basu C. Plant terpenoids: applications and future potentials. Biotechnology and Molecular Biology Reviews 2008; 3(1):1-7.
7. Kumar A, Singh S, Mahour K, Vihan VS, Guriraj K. Phytochemical Analysis of some Indigenous Plants Potent against Ectoparasite. Asian Journal of Experimental Biological Sciences. 2011; 2:506-509.
8. Savithramma N, Rao ML, Ankanna S. Preliminary phytochemical analysis of traditionally used medicinal plants. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012; 3(2):308-314.
9. Uma B, Prabhakar K, Rajendran S. *In vitro* antimicrobial activity and phytochemical analysis of *Ficus religiosa* and *Ficus bengalensis* L. against diarrhoeal enteroxigenic *E. coli*. Ethnobotanical leaflets. 2009; 13:472-474.
10. Jeremy JPE, Mohsen MMA, Minihane AM, Mathers JC. Biomarkers of the intake of diary polyphenols: strengths, limitations and application in nutrition research. British Journal of Nutrition. 2008; 99(1):12-22.
11. Abd El-Kader MA, Nafady MA, Ahmed SA, Ibraheim ZZ. Antioxidant, hepatoprotective and antimicrobial activities of the aerial parts of *Polygonum bellardii* All. Bulletin of Pharmaceutical Sciences. 2012; 35(1):43-45.
12. Kris-Etherton PM, Hecker KD, Bonanome SM, Coval AE, Binkoski KF, Hilpert-Griel AE *et al*. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine. 2002; 113:71-88.
13. Vaya J, Mahmood S, Goldblum A, Aviram M, Volkova N, Shaala A *et al*. Inhibition of LDL oxidation by

flavonoids in relation to their structure and calculated enthalpy. Phytomedicine. 2003; 62:89-99.
14. Nijveldt RJ, Nood EV, Hoorn ECV, Boelens PG, Norren KV, Leeuwen PAV. Flavonoids: a review of probable mechanisms of action and potential applications. American Journal of Clinical Nutrition 2001; 74:418-425.
15. Harsh ML, Nag TN, Jain S. Arid Zone plants of Rajasthan - A source of antimicrobials. Comparative Physiology and Ecology 1983; 8(2):129-131.
16. Jit S, Shekhawat SS, Grover S, Nag TN. Screening of some plant of Zygophyllaceae for their antimicrobial activity. Acta Botanica India 1986; 14:45-47.
17. Harborne JB, Williams CA. Advances in flavonoids research since 1992. Phytochemistry, 2000; 55:481-504.
18. Kayser O, Arndt SK. Antimicrobial activity of some *Zizyphus* species used in traditional medicine. Pharmaceutical and Pharmacological Letter. 2000; 10:38-40.
19. Baez DA, Vallejo GZ, Jimenez EZ. Phytochemical studies on *Senna skinneri* and *Senna wishizeni*. Natural Product Letters 1999; 13:223-228.
20. Xu H, Lee SF. Activity of plant flavonoids against antibiotic resistant bacteria. Phytotherapy Research 2001; 15:39-43.
21. Ogundipe OO, Moody JO, Houghton PJ, Odelola HA. Bioactive chemical constituents from *Alchornea laxiflora* (Benth.) Pax and Hoffman. Journal of Ethnopharmacology. 2001; 74:275-280.
22. Nag TN, Tyagi S, Chouhan N. Antimicrobial agent from *in-vivo* and tissue culture of arid zone cultivars. Herbal Drug and Biotechnology. Pointer publisher, Jaipur, India, 2004, 180-194.
23. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 2005; 26:343-356.
24. Stafford HA. Proanthocyanidins and the lignan connection. Phytochemistry 1988; 27:1-6.
25. Rievere C, Nguyen JHV, Pieters L, Dejaegher B, Heyden YV. Polyphenols isolated from antiradical extracts of *Mallotus metcalfianus*. Phytochemistry 2009; 70:407-419.
26. Prakash NKU, Bhuvaneswari S, Balamurugan A, Radhika B, Bhagya R. Studies on Phytochemistry of 100 plants in Chennai, India. British Journal of Pharmaceutical Research. 2013; 3:407-419.
27. Aguinaldo AM, El-Espesco BQ, Nanoto MG. Phytochemistry. In: *A* Guide Book to Plant Screening Phytochemical and Biological. Guevara, B.Q. (9th ed.), University of Santo Tomas, Manila, Philippines, 2005, 23-62.
28. Shibu A, Dhanam S. Antibacterial efficacy of leaf, stem and root powders of *Gmelina asiatica* (L.) and *Wattakakka volubilis* (L.f) Stapf. International Journal of Current Trends in Research. 2013; 2(1):100-104.
29. Parekh J, Chanda SV. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turkish Journal of Biology. 2007; 31:53-58.
30. Bakkiyaraj S, Pandiyaraj S. Evaluation of potential antimicrobial activity of some medicinal plants against common food-borne pathogenic microorganism. International Journal of Pharma and Biosciences 2011; 2(2):B484- 491.
31. Merlin NJ, Parthasarathy V, Manavalan R, Devi P, Meera R. Phyto-Physico chemical evaluation, Anti-inflammatory and Antimicrobial activities of aerial parts of *Gmelina asiatica* Linn. Asian Journal of Research in Chemistry. 2009; 2(1):76-82.
32. Sudhakar M, Rao Ch V, Rao PM. Evaluation of antimicrobial activity of *Cleome viscosa* and *Gmelina asiatica*. Fitoterapia. 2006; 77(1):47-49.
33. Merlin NJ, Parthasarathy V. Antioxidant and hepatoprotective activity of chloroform and ethanol extracts of *Gmelina asiatica* aerial parts. Journal of Medicinal plant Research. 2011; 5(4):533-538.
34. Silvia N, Satyanarayana T. Phytochemical and antioxidant studies on methanolic extract of *Gmelina asiatica* Linn. stem. International Journal of Pharmacognosy and Phytochemical Research. 2014; 6(2):276-281.
35. Kasiviswanath R, Ramesh A, Kumar KE. Hypoglycemic and antihyperglycemic effect of *Gmelina asiatica* Linn. in normal and in alloxan induces diabetic rats. Biological and Pharmaceutical Bulletin. 2005; 28(4):729-732.
36. Ikram M, Khattak SG, Gilani SN. Antipyretic studies on some indigenous Pakistani medicinal plants II. Journal of Ethnopharmacology. 1987; 19(2):185-192.
37. Azhagumurugan C, Rajan MK. Effect of leaf extract of Nilakumil, (*Gmelina asiatica*) against the root knot Nematode (*Meloidogyne Inco*gnita). Research Journal of Recent Sciences. 2014; 3:264-266.
38. Merlin NJ, Parthasarathy V. Potential Antitumour Activity of *Gmelina asiatica* Aerial Parts against Dalton Ascites Lymphoma in Mice. Asian Journal of Chemistry. 2010; 22(4):3193-3199.
39. Merlin NJ, Parthasarathy V, Santhoshkumar TR. Induction of apoptosis in human breast cancer cell line MCF-7 by phytochemicals from *Gmelina asiatica*. African Journal of Biotechnology, 2010; 9(28):4451-4456.
40. Balijepalli MK, Tandra S, Pichika MR. Antiproliferative activity and induction of apoptosis in estrogen receptor-positive and negative human breast carcinoma cell lines by *Gmelina asiatica* roots. Pharmocognosy Research 2010; 2(2):113-119.
41. Apparanantham T, Chelladurai V, Subramaniam V. Some tribal folk medicines of point calimere (Kodikkarai) in Tamil Nadu. Bulletin of Medico-Ethno-Botanical Research 1982; 3:173-177.
42. Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and methanol extracts of some medicinal for potential antibacterial activity. Turkish Journal of Biology. 2005; 29:203-210.
43. Vikneshwaran D, Viji M, Lakshmi KR. Ethnomedicinal plants survey and documentation related to Palaiyar community. Ethnobotanical Leaflets. 2008; 12:1108-1115.
44. Kusuma G, Joshi VK. Nomenclature of Anukta Dravya. Ancient Science of Life 2010; 29(4):17-23.
45. Gamble JS, Fischer CEC. Flora of Presidency of Madras. V. 1-3, Adlard and Son Ltd., London, 1935, 1-2017.
46. Harborne JB. Methods of Plant Analysis. In: Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, 1998, 1-32.
47. Evans WC. Trease and Evans Pharmacognosy. 14th edition, Bailiere Tindali, W.B. Sauders Company Ltd, London, 1996, 224-228.
48. Robert EB. Method for estimation of tannin in grain *Sorghum*. Agro Journal. 1971; 63(10):511.

49. Houghton PJ, Raman A. Laboratory Handbook for the Fractionation of Natural Extracts. 1st ed. Chapman and Hall, London, 1998, 199.
50. Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities and phenolic compounds. Journal of Agricultural Food Chemistry. 2000; 48:3567-3604.
51. Farombi EO. African plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. African Journal of Biotechnology 2003; 2:662-671.
52. Baker JT, Borris RP, Carte B, Cardell GA, Soejarto DD, Gragg GM, *et al*. Natural product drug discovery and development: New perspective in International collaboration. Journal of Natural Products 1995; 58(9):1325-1357.
53. Ahmad QR, Allen RC, Andersen TC, Anglin JD, Barton JC, Beier EW *et al*. Measurement of day and night neutrino energy spectra at SNO and constraints on neutrino mixing parameters. Physical Review Letters 2002; 89(1):011302.
54. Zafer I, Asadullah PM, Ismail M, Ahmad B, Zakir S, Saima G. Study of the hypoglycemic activity of *Hedera helix* in alloxan-induced diabetic rabbits. Journal of Medical Sciences. 2002; 2:206-208.
55. Harborne SB, Baxter H. Phytochemical Dictionary. A handbook of Bioactiove Compounds from Plants. Taylor and Francis, London, 1995, 289.
56. Rajesh NK, Silvia SP, Preethi K, Kumar E, Satyanarayana T. Pharmacognostic standardization of stem of *Gmelina asiatica* Linn. International Journal of Chemistry and Pharmaceutical Sciences. 2013; 1(3):187-192.
57. Sule WF, Okonko IO, Joseph TA, Ojezele MO, Nwanze JC. *In-vitro* antifungal activity of *Senna alata* Linn. Crude leaf extract. Advances in Applied Science Research 2010; 1:14-26.
58. Girija S, Ravindran R. Screening for qualitative Phytochemicals of *Gmelina asiatica*. Herbal Tech Industry 2011; 21:74-76.
59. Awad AB, Fink CS, Williams H, Kim U. *In vivo* (SCID mice) effects of phytosterols on the growth and dissemination of human prostate cancer PC-3 cells. European Journal of Cancer Prevention, 2001; 10(6):507-513.
60. Salminen J, Ossipov V, Haukioja E, Pihlaja K. Seasonal variation in the content of hydrolysable tannins in leaves of *Betula pubescens*. Phytochemistry 2001; 57:15-22.
61. Jaffery EH, Brown AF, Kurilich AC, Keek AS, Matusheski N, Klein BP. Variation in content of bioactive components in *Broccoli*. Journal of Food Composition and Analysis. 2003; 16:323-330.
62. Katalinic M, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry 2006; 94(4):550-557.
63. Rafat A, Philip K, Muniandy S. Antioxidant potential and content of phenolic compounds in ethanolic extracts of selected parts of *Andrographis paniculata*. Journal of Medicinal Plants Research. 2010; 4:197-202.
64. Tomas-Barberin FA, Clifford MN. Flavanones, chalcones and dihydro chalcones-nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture 2000; 80:1073-1080.
65. El-Hela A, Abdullah A. Antioxidant and Antimicrobial Activities of Methanol Extracts of some *Verbena* Species: *In vitro* Evaluation of Antioxidant and Antimicrobial Activity in relation to Polyphenolic Content. Journal of Applied Sciences Research. 2010; 6(6):683-689.
66. Macheix JJ, Fleuriet A, Billot J. Fruit Phenolics. CRC Press: Boca Raton, FL, USA, 1990, 106-107.
67. Randhir Y, Lin T, Shetty K. Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. Asian Pacific Journal of Clinical Nutrition. 2004; 13:295-307.