ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Comparative Quantitative Screening of Secondary Phytoconstituents from the leaves extract of *Sterculia foetida* Linn

Jeyabaskar Suganya[1]*, Viswanathan T[2], Mahendran Radha[1], Rathisre P.R[1], Nishandhini Marimuthu[1]

[1]Department of Bioinformatics, School of Life Sciences, Vels University, Pallavaram, Chennai-17. Tamil Nadu, India.

[2] Department of Microbiology, LRG Govt. Arts College of Women, Tiruppur- 641 604, Tamil Nadu, India.

*Corresponding Author E-mail: **suganyaj11@gmail.com**

ABSTRACT:

In developing countries like India, more than 60 percentage of the population relies only on the traditional medicine in order to meet their physical conditions. Traditional medicine has been more commonly practiced in ayurvedic treatment without knowing the knowledge of the actual secondary constituents present in the plant material. Secondary constituents present in the plants play an important role in healing and curing various human diseases. The first and foremost step in *in vitro* drug designing was the screening of phytoconstituents of the traditional plants. Through literature survey on traditional plant, *Sterculia foetida* was a frequently used plant for its high medicinal properties. To our knowledge, this current investigation was the first attempt to screen the amount of phytoconstituents present in the leaves of *Sterculia foetida* using five different solvent (hexane, chloroform, methanol, ethyl acetate and aqueous) based on their polarity. On comparative studies of the current research, the results revealed that the methanol leaves extracted exhibit a high yield percentage of secondary constituents when compared with other four solvents. Further identification, isolation of biocompounds present in the methanol leaves could lead to a novel natural antioxidant which was used in preventing various diseases.

KEYWORDS: *Sterculia foetida* Linn, Leaves, Five solvents, Quantative, Secondary phytoconstituents.

INTRODUCTION:

Plants play a vital part in nature. Nature is gifted with a unique source of high photochemical with better biological activities and medicinal properties[1]. The WHO reported that up to 80% of the human population in the developing countries mainly relies on traditional medicine as their primary treatment[2]. Nowadays, usage of traditional medicines is increased and also gets popularity over the developed countries than synthetic medicines.

Traditional plants are a rich source of novel drugs molecules which can be utilized for modern medicines, pharmaceutical intermediates and lead compounds in natural drugs[3, 4]. In traditional plants, these natural compounds occur in the leaves, barks, vegetables, fruits, roots and even stem which function as the defense mechanism in human bodies and protects from various diseases[5].

The cells present in the plant has the capacity to produce primary metabolites and secondary metabolites[6, 7]. The primary metabolites are responsible for the growth and development where as the secondary metabolites are responsible for defense mechanism in the plants[8] and also for some metabolic activities[9]. The plant secondary metabolites can be used as fibers, oils, flavoring agents, perfumes, and also sight as potential sources of new natural antibiotics and insecticides[10]. The three most

Received on 07.03.2017 Modified on 22.03.2017
Accepted on 14.04.2017
Research J. Pharm. and Tech. 2017; 10(9): 2907-2912.
DOI: 10.5958/0974-360X.2017.00513.3

important secondary metabolites flavonoids, tannin, phenols and some other metabolites like saponin, alkaloids, and cardiac glycosides are also present in the plant cells[11,12]. The plant compounds fall under the flavonoids group possesses diverse biological functions which includes the protection of plants against UV radiation and phytopathogens[13]. In many plants, they are also responsible for the fall of color, which result in the protection of leaf cells from photooxidative damage and for the coloration of flowers, finally solving the requirement of nutrient during senescence[14]. The phenol compounds work as antimicrobial metabolites and also play a role for lignin, pigment biosynthesis and reproduction of the plant[15]. Plants containing tannin compounds have various properties like astringent, hemostatic, antiseptic and toning[16].

The traditional plant *Sterculia foetida* is a deciduous tree which grows straight and large upto 40m in height and 3m in girth[17]. The native of *Sterculia foetida* is Eastern Asia (Cambodia, India, Malaysia, Myanmar, Sri Lanka, and Thailand) and it is called in tamil as pottaikavalam, gurapu-vadam and gorapu-badam. The branches of the tree are arranged in whorls and spreads horizontally. The traditional plant is reported to possess various pharmacological properties and phytoconstituents present in the plant exhibits several biological properties like antifungal, antimicrobial, antiviral, antitumor and anticancer activities[18, 19]. In olden days, the leaves of the plant were used as medicine without the knowledge of their medical properties[20]. The leaves of *Sterculia foetida* are located at the ends of each branchlets with 7-9 leaflets. Each leaflet grows elliptically upto 10–17 cm with the petiole of 12.5–23 cm long[21] were shown in Table 1. The earlier research on leaves predicted that they are a good source of protein, phosphorus and calcium which solve the dietary requirements for ruminants.

Table 1: Scientific Classification of *Sterculia foetida* L.

Kingdom	**Plantae**	**Plants**
Subkingdom	Tracheobionta	Vascular plants
Super-division	Spermatophyta	Seed plants
Division	Magnoliophyta	Flowering plants
Class	Magnoliopsida	Dicotyledons
Subclass	Dilleniidae	
Order	Malvales	
Family	Sterculiaceae	Cacao family

Fig. Leaves of *Sterculia foetida* L.

The medicinal properties of the leaves of *Sterculia foetida* mainly relies on the presence of important secondary metabolites (Flavonoids, phenols, tannins) which perform as a natural antioxidant source to human beings. The aim of the present investigation was to reveal the amount of secondary metabolites present in leaves extract of five different solvent like hexane, chloroform, methanol, ethyl acetate and aqueous using standard protocols.

MATERIALS AND METHODS:

Selection of leaves of *S.foetida*:

The disease free, mature and fresh leaves of *S. foetida* were collected from Pallavaram of Chennai District, Tamil Nadu (Fig.2). The leaves were washed thoroughly with running tap water for 2-3 times to remove the dust present in the leaves. Then the leaves were air dried at room temperature for 2 weeks and then made into coarse powder using electric blender and stored in clean closed bottles for further quantitative analysis.

Authentication of the leaves of *S. foetida*:

The leaves of *S. foetida* were authenticated by Dr. D. Narasimhan Ph.D. in Botany. Medicinal plants, Associate professor, Department of Botany, Madras Christian College (Autonomous), Tambaram.

Extraction of the leaves through soxhlet:

The 100 grams of powdered leaves were kept overnight in 5 different solvents systems and extraction were performed. Every time before extracting the leaves with the next solvent, the current extract was dried. Based on their increased polarity, the five different solvents like hexane, chloroform, methanol, ethyl acetate and aqueous were selected for the extraction of bioactive compounds by continuous percolation process using soxhlet apparatus.[22]

Evaporation of the leaves through rotary evaporator:

After extraction using soxhlet, the collected individual solvent extracts were dried separately using rotary vacuum evaporator under reducing pressure and temperature. Then the extracts were allowed to cool and small amount of the extracts were subjected to lyophilization. The collected extracts of each solvent were weighed and its percentage yield was calculated in terms of dried weight of the leaves material. The appearance and consistency of each extracts were recorded. Finally, each extracts were ready for further evaluation of the quantitative parameters.[23]

Quantitative screening of secondary phytoconstituents:

Secondary phytoconstituents mainly fall under three classes of compounds; they are flavonoids, phenols and tannin. Lakhs and thousands of secondary phytoconstituents have been identified and isolated from

the plants materials. Many of the phytoconstituents and their derivatives has the capacity to play powerful physiological effects in human life. Over the last century, phytoconstituents play a very significant role in the treatment of various diseases and further can be used as medicines. Secondary phytoconstituents are important mediators of ecological interactions in plant defence mechanism.

Estimation of total flavonoids content using Aluminum chloride colorimetric method: [24, 25]

The solution of 1 mg/ml of five different leaves extract of *S.foetida* was mixed with 3 ml of methanol followed by 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and finally by 5.6 ml of distilled water. The mixtures of the solution were kept at room temperature for 30 minutes for the reaction to take place. The standard gallic acid solution (0-0.8 mg/ml) in distilled water was prepared separately. The absorbance of the leaves solution and the standard was measured at 420 nm using UV visible spectrophotometer. The amount of flavonoids present in the different extracts was determined from extrapolation of calibration curve. The concentration of flavonoids present in the five different leaves extract was expressed in terms of mcg/mg**.**

Estimation of total phenols content using Slinkard and Singleton method: [26, 27]

The 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of (2% w/v) Na_2CO_3 were added to 0.5 ml of 1 mg/ml five different leaves extract of *S. foetida*. The resulting solution was incubated at 45°C with continuous shaking for 15 minutes. The standard gallic acid solution (1mcg/mg) in distilled water was prepared separately. The absorbance o of the leaves solution and standard was measured at 765 nm with UV/visible light. The amount of phenols present in the different extracts was determined from extrapolation of calibration curve. The concentration of phenols present in five different leaves extract were expressed in terms of mcg/mg**.**

Estimation of total tannins content using Folin-Denis reagent: [28, 29]

The solution of 0.5gm of five different leaves extract of *S.foetida* was boiled with 5 ml distilled water. Then the extraction from the leaves were centrifuged at 5, 000 rpm for 10 minutes. The supernatant was collected separately. 1 ml of the supernatant was added to 0.5 ml Folin-Denis reagent and 1 ml of Na_2CO_3 solution. The volume of the extraction of leaves was finally adjusted to 10 ml with distilled water. The reaction solution was incubated for 30 minutes at room temperature. The standard tannic acid solution in distilled water was prepared separately. The absorbance of the leaves solution and standard was measured at 775 nm on UV/visible light. The amount of tannins present in the different extracts was determined from extrapolation of calibration curve. The concentration of tannins was expressed in mcg/mg**.**

RESULT AND DISCUSSION:

Yield of leaves extract:

In the current study, the five extracts (Hexane, Chloroform, Ethyl acetate, Methanol and Aqueous) of *Sterculia foetida* were prepared by the solvent extraction method. The yield percentage of crude extracts was obtained by measuring its dryness per 100 grams of extract. The yield percentage was found to be more in Aqueous (12.35%) followed by methanol (7.25%) and the yield percentage was found to be less in hexane (1.39%). The consistency of the extract was found in the form of powder in chloroform and paste in other solvents (Table 2)[30]

Table 2: Quantitative yield of phytoconstituents from leaves extract of *Sterculia foetida*

S. No	Solvent	Yield (%) Per 100 g	Consistency
1	Hexane	1.39	Paste
2	Chloroform	2.41	Powder
3	Ethyl acetate	3.87	Paste
4	Methanol	7.25	Paste
5	Aqueous	12.35	Paste

Quantitative estimation of total flavonoids content:

The presences of flavonoids were observed in the all five leaves extract of *Sterculia foetida* in all concentrations. This result confirmed that the presence of high quantitative of flavonoids in the leaves of *Sterculia foetida*. On further analysis, the total flavonoids present in the leaves were shown high in the methanol extract of *Sterculia foetida* (48.83) in 100µg concentrations followed by chloroform (37.47), ethyl acetate (22.79), aqueous (12.13), hexane (11.21) extracts (Table 3 and Fig1). The presence of flavonoids in the leaves of *Sterculia foetida* may exhibit various pharmacological activities like antioxidants, anti-inflammatory, anti-tumour, and antibacterial, antiviral and anti-allergic[31]. The flavonoids may act as inhibitor of lipid-peroxidation, platelet aggregation, cAMP phosphodiesterase, lipase, α-glucosidase, kinase, cyclo-oxygenase and lipoxygenase enzyme activities.[32]

Table 3: Quantitative Analysis of Total Flavonoid Assay of different Leaves Extracts *Sterculia foetida*

S. No	Concentration (µg)	Methanol	Chloroform	Ethyl Acetate	Hexane	Aqueous
1	60	36.45	21.27	16.37	9.83	9.26
2	80	44.01	32.89	21.53	10.98	10.75
3	100	48.83	37.47	22.79	11.21	12.13

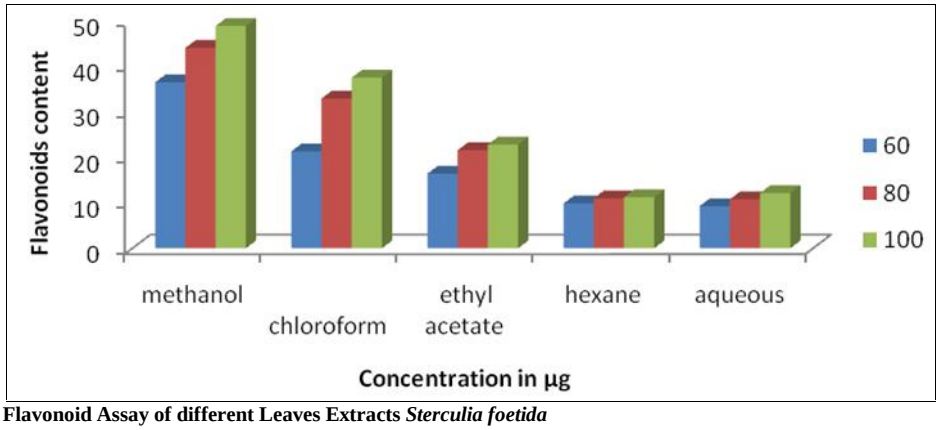

Figure 1: Total Flavonoid Assay of different Leaves Extracts *Sterculia foetida*

Quantitative estimation of total phenolic content:

The presence of phenol was observed in Methanol, Chloroform, Hexane and Aqueous leaves extract of *Sterculia foetida* in concentrations of 80 µg and 100 µg (Table 4 and Fig 2) whereas ethyl acetate extract showed the absence of phenol in all concentrations. On analyzing the results, further it was predicted that the methanol extract exhibited high percentage of phenol in all concentrations. Chloroform, Hexane and Aqueous exhibited a good percentage in maximum concentrations and trace percentage in the lower concentrations. The presence of phenol in the leaves of *Sterculia foetida* plays an important role in the regulation of plant growth, development and disease resistance[33]. When the plant polyphenols were consumed in the human diet, phytochemical may act as a barrier for the development of cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases. [34]

Table 4: Quantitative Analysis of Total Phenol Assay of different Leaves Extracts *Sterculia foetida*

S. No	Concentration (µg)	Methanol	Chloroform	Ethyl Acetate	Hexane	Aqueous
1	80	20.94	6.94	-	2.83	3.01
2	100	32.24	12.34	-	10.08	8.53

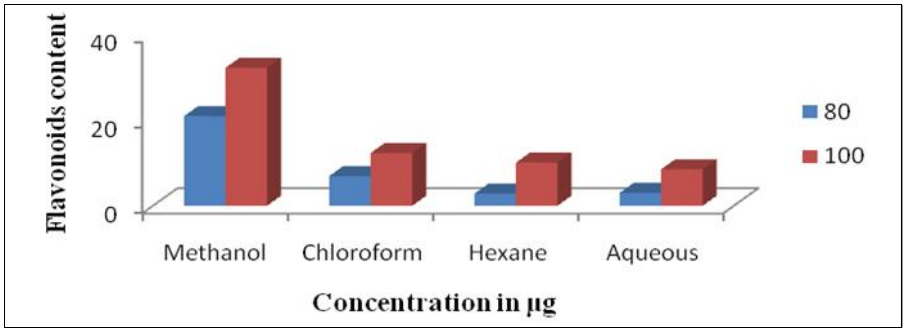

Figure 2: Total phenolic Assay of different Leaves Extracts *Sterculia foetida*

Quantitative estimation of total Tannin content:

The presence of high quantative of Tannin was observed in the all five leaves extracts of *Sterculia foetida* in all three (60µg, 80µg and 100µg) concentrations. (Table 5 and Fig 3). On comparative analysis, the results obtained are as follows: the methanol extract exhibited high content of tannin even at low concentration (88.79) and in maximum concentration (131.54). The second high content of tannin were observed in hexane extract (112.38 at 100µg)) and followed by aqueous extract (101.08 at 100µg). The chloroform and ethyl acetate extract exhibited good quantative of tannin in (79.95, 54.38) 100µg concentration. The presence of tannin in the leaves of *Sterculia foetida* contributes various medicinal properties like anti-viral, antibacterial, anti-parasitic antimicrobial, anti-inflammatory and astringent activity. [35]

Table 5: Quantitative Analysis of Total Tannin Assay of different Leaves Extracts *Sterculia foetida*

S.No	Concentration (µg)	Methanol	Chloroform	Ethyl Acetate	Hexane	Aqueous
1	60	88.79	20.49	17.54	69.14	51.94
2	80	110.90	26.38	32.28	83.39	70.61
3	100	131.54	79.95	54.38	112.38	101.08

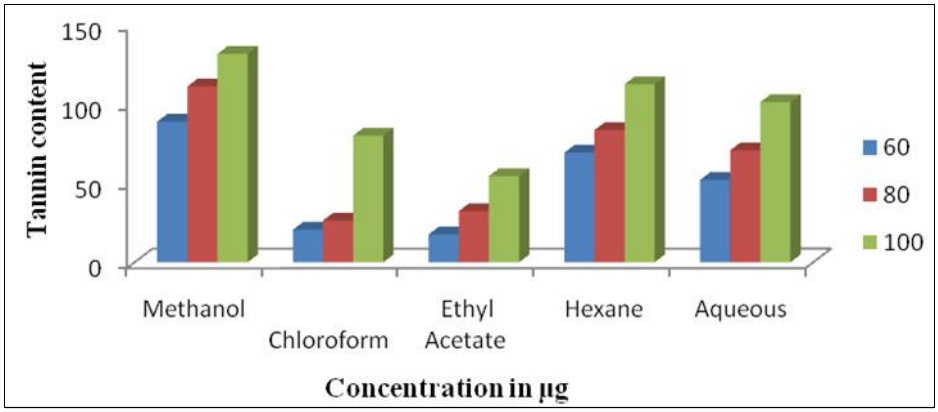

Figure 3: Total Tannin Assay of different Leaves Extracts *Sterculia foetida*

CONCUSION:

Traditional medicines are free from adverse and side effects. Over the centuries they are economical, easily available drugs for mankind. The medicinal values of traditional plants mainly lie in some phytoconstituents, which produce biological action over human bodies when they are consumed. In the current investigation, the quantitative screening of secondary phytoconstituents present in the five different leaves extracts of *Sterculia foetida* were evaluated. On comparative screening of the extraction of leaves, the results explored that the methanol leaves extract showed better existence of secondary phytoconstituents than other solvent extracts. On further analysis of secondary phytoconstituents in methanol extract revealed that the tannin compounds found to exhibit in high yield percentage of amount in the leaves and followed by the flavonoids compounds with good yield percentage and next with phenol compounds in average percentage. Thus, screening of phytoconstituents leaves of *Sterculia foetida* played a very important role in identifying a new source of valuable compounds from traditional plants in both therapeutic and industrial purposes.

CONFLICT OF INTEREST:

The authors declare they have no competing interests.

ACKNOWLEDGEMENT:

We acknowledge Vels Institute of Science, Technology and Advanced Studies (VISTAS) for providing us with required infrastructure and support system needed.

REFERENCES:

1. Angeline Christie Hannah M, Krishnakumari S. Quantitative estimation of plant metabolites in the hot aqueous seed extract of watermelon (*Citrullus vulgaris Schrad*.) Journal of Medicinal Plants Studies 2015; 3(5): 107-111
2. Martins Ekor. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2013; 4(177): 1-10
3. Renuga Devi M, Krishnakumari. S Quantitative estimation of primary and secondary metabolites in hot aqueous extract *of Pleurotus sajor caju*. Journal of Pharmacognosy and Phytochemistry 2015; 4(3): 198-202
4. Saleh Hosseinzadeh, Azizollah Jafarikukhdan, Ahmadreza Hosseini, Raham Armand. The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of *Thymus vulgaris*. International Journal of Clinical Medicine. 2015; 6: 635-642.
5. Gnanaraja R, Veeru Prakash, Shanta Peter and Mahendraverman M. Qualitative and quantitative phytochemicals analysis of selected fabaceae medicinal plants from Allahabad region. The Pharma Innovation Journal 2014; 3(7): 53-56.
6. Md. Sarfaraj Hussain, Sheeba Fareed, Saba Ansari, Md. Akhlaquer Rahman, Iffat Zareen Ahmad,1 and Mohd. Saeed1. Current approaches toward production of secondary plant metabolites. J Pharm Bioallied Sci. 2012; 4(1): 10–20.
7. Mohd Hafiz Ibrahim, Hawa Z E Jaafar, Ehsan Karimi, and Ali Ghasemzadeh . Primary, Secondary Metabolites, Photosynthetic Capacity and Antioxidant Activity of the Malaysian Herb Kacip Fatimah (*Labisia Pumila* Benth) Exposed to Potassium Fertilization under Greenhouse Conditions. Int J Mol Sci. 2012; 13(11): 15321–15342.
8. Clemencia M. Rojas, Muthappa Senthil-Kumar, Vered Tzin,1,3 and Kirankumar S. Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. Front Plant Sci. 2014; 5(17): 1-12.
9. Mazid M, Khan TA, Mohammad F. Role of secondary metabolites in defense mechanisms of plants. Biology and Medicine. 2011; 3 (2): 232-249,
10. Michael Wink. Modes of Action of Herbal Medicines and Plant Secondary Metabolites. Medicines 2015; 2: 251-286;
11. Faten Medini, , Hanen Fellah, Riadh Ksouri, Chedly Abdelly. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. Journal of Taibah University for Science, 2014; 8 (3): 216–224.
12. Dr. Raghuveer Irchhaiya, Anurag Kumar, Anumalik yadav, Nitika Gupta, Swadesh Kumar, Nikhil Gupta, Santosh Kumar, Vinay Yadav, Anuj Prakash2 and Himanshu Gurjar. Metabolites in plants and its classification. World Journal of Pharmacy and Pharmaceutical Sciences. 2015; 4 (1): 287-305.
13. Justyna Mierziak, Kamil Kostyn, and Anna Kulma. Flavonoids as Important Molecules of Plant Interactions with the Environment. Molecules. 2014; 19: 16240-16265.
14. María L. Falcone Ferreyra, Sebastián P. Rius, and Paula Casati. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Front Plant Sci. 2012; 3 (222): 1-15.
15. Amita Bhattacharya, Priyanka Sood, Vitaly Citovsky the roles of plant phenolics in defence and communication during Agrobacterium and Rhizobium infection. Molecular Plant Pathology. 2010; 11(5): 705-19.

16. Praveen Kumar Ashok, Kumud Upadhyaya. Tannins are Astringent. Journal of Pharmacognosy and Phytochemistry. 2012; 1(3): 45- 50.
17. Abdulaziz Atabani, Mahlia T.M.I, Irfan Anjum Badruddin, Lee K.T. Investigation of physical and chemical properties of potential edible and non-edible feed stocks for biodiesel production, a comparative analysis. Renewable and Sustainable Energy Reviews. 2013; 21: 749-755.
18. Schmid KM, Patterson GW. Effects of cyclopropenoid fatty acids on fungal growth and lipid composition. Lipids. 1988; 23(3): 248-252.
19. Salaun J. Cyclopropane derivatives and their diverse biological activities. Topics in Current Chemistry. 2000; 207: 1-67.
20. Jin-Ming K, Ngoh-Khang G, Lian-Sai C, Tet-Fatt C. Recent advances in traditional plant drugs and orchids. Acta Pharmacologica Sinica. 2003; 24(1): 721-731.
21. Prakash Yoganandam G, Gopal V, Kaviarasan L. Promising pharmaceutical prospective of 'Java Olive'- *Sterculia foetida Linn* (Sterculiaceae). International Journal of Pharmacy Review and Research. 2012; 2(2): 93-96.
22. Andri Cahyo Kumoroa, Masitah Hasana, Harcharan Singha, Effects of solvent properties on the Soxhlet extraction of diterpenoid lactones from Andrographis paniculata leaves. Science Asia. 2009; 35: 306–309.
23. Mohammad Amzad Hossain, Seham Salim Al-Hdhrami, Afaf Mohammed Weli, Qasim Al-Riyami, and Jamal Nasser Al-Sabahi. Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of *Mentha piperita* L grown in Sultanate of Oman. Asian Pac J Trop Biomed. 2014; 4(1): S368–S372.
24. Durai MV, Balamuniappan G, Anandalakshmi R, Geetha S and Senthil Kumar N. Qualitative and quantitative analysis of phytochemicals in crude extract of big – Leaf mahogany (*Swietenia macrophylla* King). International Journal of Herbal Medicine 2016; 4(6): 88-91.
25. Kalita Pallab, Barman Tapan K, Pal Tapas K, Kalita Ramen. Estimation of total flavonoids content (TFC) and anti-oxidant activities of methanolic whole plant extract of *Biophytum sensitivum* linn. Journal of Drug Delivery and Therapeutics. 2013; 3(4): 33-37
26. Afsaneh Moslemi Mehni, Fatemeh Shahdadi. Phenolic compounds and antiradical properties of methanolic extracts of *Citrullus colocynthis* and *Plantago major* in Iran. Int. J. Biosci. 2014; 4 (3): 224-228.
27. Mahfuz Elmastaş, Sed Çinkiliç, Hassan Y. Aboul-Enein. Antioxidant Capacity and Determination of Total Phenolic Compounds in Daisy (*Matricaria chamomilla, Fam. Asteraceae*). World Journal of Analytical Chemistry. 2015; 3 (1A): 9-14.
28. Vasundhara Saxena, Garima Mishra, Akash Saxena. Kamlesh KR. Vishwakarma. A comparative study on quantitative estimation of tannins in *Terminalia Chebula, Terminalia Belerica, Termina;lia Arjuna* and *Saraca Indica* using spectrophotometer. Asian J Pharm Clin Res. 2013; 6(3): 148-149.
29. Durgawale Trupti Pratik, Durgawale Pratik Prakash, Khanwelkar Chitra Chandra shekhar. Quantitative Estimation of Tannins from Pueraria tuberosa by UV Spectrophotometry. Int. J. Pharm. Sci. Rev. Res. 2016; 37(1): 210-212.
30. Mohamed El Hasan Shayoub, Azza Dawoud Hussien Dawoud, Mona AM Abdelmageed, Ali M Ehassan, Ahmad M Ehassan. Phytochemical analysis of leaves extract of Eucalyptus *camaldulensis* Dehnh. Omdurman Journal of Pharmaceutical Science. 2015; 2(1): 64-71.
31. Madhu M, Sailaja V, Satyadev TNVSS, Satyanarayana MV. Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. Journal of Pharmacognosy and Phytochemistry 2016; 5(2): 25-29.
32. Harleen Kaur Sandhar, Bimlesh Kumar, Sunil Prasher, Prashant Tiwari, Manoj Salhan, Pardeep Sharma. A Review of Phytochemistry and Pharmacology of Flavonoids. Internationale Pharmaceutica Sciencia. 2011; 1(1): 25-41.
33. Santi M. Mandal, Dipjyoti Chakraborty and Satyahari Dey. Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signaling and Behavior. 2010; 5(4): 359-368.
34. Magdalena Działo, Justyna Mierziak, Urszula Korzun, Marta Preisner, Jan Szopa and Anna Kulma. The Potential of Plant Phenolics in Prevention and Therapy of Skin Disorders. Int. J. Mol. Sci. 2016; 17(160): 1-41.
35. Abd E-Moneim MR Afify, Hossam S El-Beltagi, Samiha M Abd E-Salam, and Azza A Omran. Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. Asian Pac J Trop Biomed. 2012; 2(3): 203–209.