



## Screening of Secondary Metabolites of Underutilized Species of Cyperaceae

R. Hari Babu<sup>\*1</sup>, Dr. N. Savithamma<sup>2</sup>

<sup>\*1</sup>Research Scholar, Department of Botany, S.V. University, Tirupati, Chittoor (Dt), Andhra Pradesh, India.

<sup>2</sup>Professor, Department of Botany, Sri Venkateswara University, Tirupati, Chittoor (Dt), Andhra Pradesh, India.

\*Corresponding author's E-mail: [harisan82@gmail.com](mailto:harisan82@gmail.com)

Accepted on: 23-11-2013; Finalized on: 31-01-2014.

### ABSTRACT

Phytochemicals are important constituents of plants to improve economic values of species. These studies were neglected in the members of cyperaceae. Hence the aim of the study to screen the South Indian grass plants of cyperaceae for phytochemical constituents. The results obtained in the present study suggest that preliminary phytochemical analysis detuned the presence of Alkaloids, Coumarins, Flavonoids, Glycosides, Lignins, Phenols, Quinones, Saponins, Steroids, Tannins and Terpenoids. Results of quantitative phytochemical analysis revealed that high content of Alkaloids, followed by Phenols, Tannins, Saponins and Flavonoids receptively. The results suggest that the plant species posses the properties for curing various ailments and may be the source for potential antioxidant, anti-inflammatory and antimicrobial activities. Which may leads to isolation of new and novel compounds.

**Keywords:** Cyperaceae and Phytochemicals, Grasses, Secondary metabolites.

### INTRODUCTION

The grasses that yield food and fodder are well known from times immemorial and considerable amount of research work has been carried on them. But the grasses that yield therapeutically important products are among the least studied in the cyperaceae family. Phytochemicals are naturally occurring, biologically active chemical compounds in plants. They act as a natural defense system for host plants and provide colour, aroma and flavor. These are non-nutritive plant chemicals that have protective or disease preventive properties.<sup>1</sup> Many antioxidant compounds naturally occurring in plant source have been identified as free radicals or active oxygen scavengers.<sup>2</sup> Phytochemicals like carotenoids, flavonoids and polyphenols, they posses antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. The majority of the active compounds are phenolics, vitamin C, vitamin E, tannins and carotenes.<sup>3</sup> Sources of natural antioxidants are primarily plant phenolics such as flavonoids that exhibit antioxidant, antimicrobial, anti carcinogenicity and other biological activities.<sup>4</sup>

World plant biodiversity is the largest source of herbal medicine and still about 60-80% world population rely on plant based medicines which are being used since the ancient ages as traditional health care system. It is now clear that, the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. These natural compounds formed the base of modern drugs as we use today.<sup>5</sup> Cyperaceae is a family of monocotyledonous graminoid flowering plants known as sedges, which superficially resemble grasses. The family is large, with 5,500 species described. In about 109 genera

worldwide although estimates of numbers vary greatly due to differing taxonomic concepts of individual researches and because modern sedge floras are available for only a few countries. The Chief importance of sedges lies in their forming a major natural constituent of wetlands and river side vegetation, where their densely tangled rhizomes contribute to erosion control and water purification. While on the natural theme, the dense sedge beds that form in swampy regions provide food and shelter for bird's animals and other aquatic life thus attracting ecosystem. These grasses are included in traditional medicine system but yet have to be explored. Hence the aim of this study is to examine the phytochemical composition of aqueous extracts of ten cyperaceae grass species. Preliminary phytochemical screening of the plants is primarily an important aspect in finding the chemical constituents in plants.

Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humen based on their toxicity against cells of foreign organisms. Therefore, the compounds detected may be responsible for the antibacterial activity of the herb extracts. Flavonoids exhibited a wide range of biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-angionic, anticancer and anti-allergic.<sup>6,7,3</sup> The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, anti allergic, anti platelet, anti inflammatory, antitumor, anti carcinogenic and anti ageing properties.<sup>8,9</sup> Flavonoids may help provide protection against these diseases by contributing along with antioxidant vitamins and enzymes. Flavonoids are free radical scavenger, super antioxidant and potent water soluble which prevent oxidative cell damage and have strong anti cancer



activity.<sup>10</sup> Flavonoids apart from their antioxidant protection effects, inhibit the initiation, promotion and progression of tumour.<sup>10 11</sup> Saponins which are one of the active constituents involved in plant disease resistance because of their antimicrobial activity.<sup>12</sup> Traditionally, saponins are subdivided into triterpenoid and steroid glycoside. Tannins are phenolic compound which act as primary antioxidants or free radical scavengers.<sup>13</sup> According to,<sup>14</sup> several phenolic compounds like tannins present in cells of plant are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. In addition, herbs that has tannins as their main components are astringent in nature.

## MATERIAL AND METHODS

### Collection and identification of plant material

Fresh leaves of *Bulbostylis barbata*, *Cyperus difformis*, *Cyperus rotundus*, *fimbristylis monostachya*, *Fimbristylis cymosa*, *Fimbristylis eragrostis*, *Kyllinga monoceps*, *Kyllinga triceps*, *Paspalidium flavidum* and *Scelaria lithosperma* were collected from the different locations of South India, during the month of December, 2011. The leaves were washed thoroughly 2-3 times with running tap water, leaf material was then air dried under shade after complete shade drying the plant material was used for phytochemical analysis.

### Preparation of extract

The grinded leaf material of 5g was crushed in 25 ml of sterile water, boiled at 50-60°C for 30 minutes on water bath and it was filtered through What man No. 1 filter paper. Then filtrate was centrifuged at 2500 rpm for 15 minutes and filtrate was stored in sterile bottle at 5°C for further use.<sup>15</sup>

## Preliminary screening and quantification of phytochemicals

The condensed extracts were used for preliminary screening of phytochemicals by following the methods for alkaloids, lignin, phenols and steroids;<sup>16</sup> Coumarins and flavonoids;<sup>17</sup> glycosides, terpenoids and saponins<sup>13</sup> and tannins.<sup>18</sup> Quantification of alkaloids and saponins;<sup>15</sup> flavonoids and phenols and tannins.<sup>19 20</sup>

## RESULTS AND DISCUSSION

Qualitative phytochemical studies revealed that the aqueous extract of plant species contained phytoconstituents like Alkaloids, Coumarins, Flavonoids, Glycosides, Lignins, Phenols, Quinones, Saponins, Steroids, Tannins and Terpenoids (Table 1). Six species of selected plants possess Alkaloid compounds in aqueous extracts. The highest levels of alkaloids were found in *Cyperus rotundus* 0.810 mg/g dw followed by *Scelaria lithosperma* (Table 2). Alkaloids are found in *Cymbopogon* and *Cynodon dactylon*.<sup>21,22</sup> Most of the plants have alkaloids in different organs with different chemical configurations.<sup>23</sup> Alkaloids are beneficial chemicals to plants with predator and parasite repelling and physical state. Number of alkaloids was isolated from dicots and using as efficient drugs. Coumarins are present in three plants among selected ten grasses, which reduce the impact of grazing animals. Though the compound has a pleasant sweet odor, it has a bitter taste, and animals tend to avoid it. Reports on coumarin activity includes anti-HIV, anti-tumor, anti-hypertension, anti-arrhythmia, anti-inflammatory, anti-osteoporosis, antiseptic and analgesic. It is also used in the treatment of asthma and lymphedema.<sup>24</sup>

**Table 1:** Preliminary phytochemical screening aqueous extract of ten selected cyperaceae grass species

Name of the species	Alkaloids	Coumarins	Flavonoids	Glycosides	Lignin's	Phenols	Quinones	Saponins	Steroids	Tannins	Terpenoids
<i>Bulbostylis barbata</i>	-	-	+	-	-	+	+	+	-	-	-
<i>Cyperus difformis</i>	-	-	-	+	+	-	+	-	-	+	+
<i>Cyperus rotundus</i>	+	+	+	-	+	+	-	+	+	+	-
<i>Fimbristylis cymosa</i>	-	-	+	+	+	-	-	+	-	-	-
<i>Fimbristylis eragrostis</i>	+	-	+	-	-	+	+	+	-	-	-
<i>fimbristylis monostachya</i>	+	-	+	-	-	-	-	+	+	+	-
<i>Kyllinga monoceps</i>	-	+	-	-	-	-	+	+	+	+	+
<i>Kyllinga triceps</i>	+	-	+	+	+	+	-	-	+	-	-
<i>Paspalidium flavidum</i>	+	+	+	+	-	-	+	-	-	+	-
<i>Scelaria lithosperma</i>	+	-	-	-	+	+	-	+	+	+	-

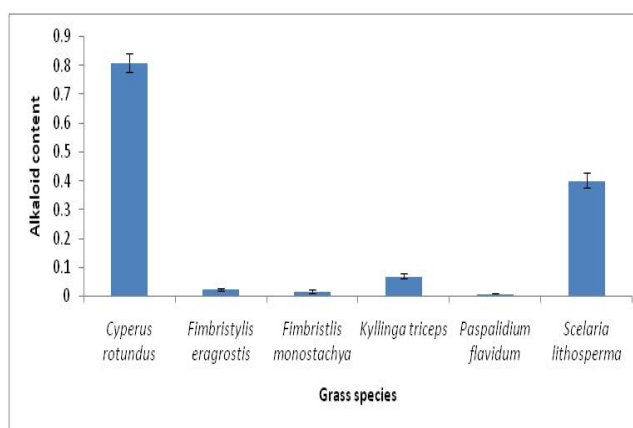
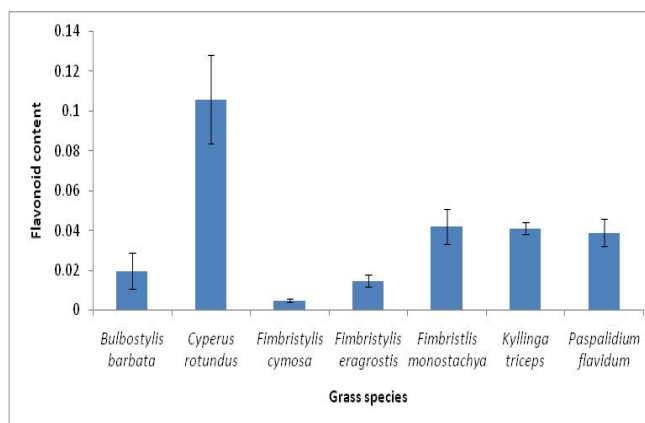
**Note:** '+' indicates presence and '-' indicates absence.

**Table 2:** Quantification of secondary metabolites from selected cyperaceae grass species (mg/g dw)

Name of the species	Alkaloids	Flavonoids	Phenols	Saponins	Tannins
<i>Bulbostylis barbata</i>	-	0.020 ± 0.009	0.200 ± 0.018	0.020 ± 0.011	-
<i>Cyperus difformis</i>	-	-	-	-	0.005 ± 0.001
<i>Cyperus rotundus</i>	0.810 ± 0.033	0.106 ± 0.022	0.630 ± 0.041	0.318 ± 0.042	0.423 ± 0.038
<i>Fimbristylis cymosa</i>	-	0.005 ± 0.001	-	0.004 ± 0.001	-
<i>Fimbristylis eragrostis</i>	0.024 ± 0.004	0.015 ± 0.003	0.082 ± 0.011	0.013 ± 0.002	-
<i>Fimbristylis monostachya</i>	0.019 ± 0.007	0.042 ± 0.009	-	0.009 ± 0.002	0.033 ± 0.007
<i>Kyllinga monoceps</i>	-	-	-	0.008 ± 0.003	0.217 ± 0.004
<i>Kyllinga triceps</i>	0.070 ± 0.009	0.041 ± 0.003	0.007 ± 0.002	-	-
<i>Paspalidium flavidum</i>	0.010 ± 0.001	0.039 ± 0.007	-	-	0.028 ± 0.007
<i>Scelaria lithosperma</i>	0.401 ± 0.026	-	0.136 ± 0.034	0.240 ± 0.002	0.106 ± 0.003

Among ten selected plants seven species possessing Flavonoids. *Cyperus rotundus* containing maximum levels of flavonoids followed by *Fimbristylis monostachya*. Flavonoids are recorded in the grass of *Cyperus rotundus*,<sup>25</sup> *Cynodon dactylon*,<sup>21</sup> *Saccharum spontaneum*,<sup>26</sup> *Imperata cylindrical*,<sup>27</sup> *Pennisetum purpureum* and *Cymbopogon citrates*.<sup>28 29</sup> They also inhibit microbes which are resistance to antibiotics.<sup>30</sup> Flavonoids are free radical scavengers and have strong anti-cancer activity<sup>31</sup> evidence of their inherent ability to modify the body's reaction to allergies, virus and carcinogens. flavonoid rich species can play the role in pharmacological activities as anti-inflammatory, analgesic, anti-oxidant, antifungal and immunostimulant providing a key role of flavonoids to their biological actions.<sup>8</sup> Glycoside substances are absent in six grasses and found in four grasses among selected ten species. They are important in medicine because of their action on heart and are used in cardiac insufficiency.<sup>32</sup> Lignins are present in five species of selected plants. Lignins are significant components in the global carbon cycle; the resistance of lignin to microbial degradation enhances its persistence in soils.<sup>33</sup>

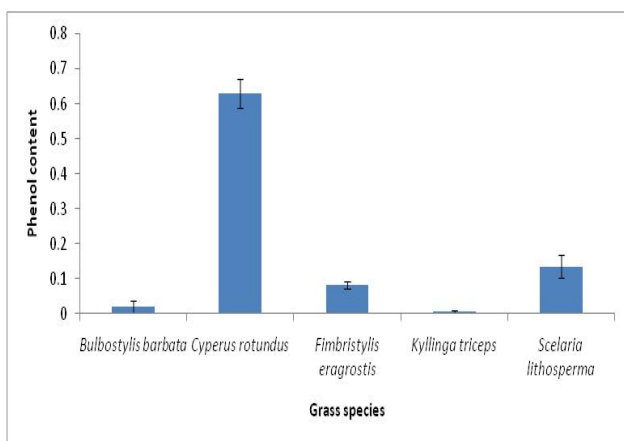
Five species among ten selected grasses possess phenol compounds. *Cyperus rotundus* is the rich source of phenol compounds followed by *Bulbostylis barbata*.<sup>21 29 27</sup> studied the phenols in *Cymbopogon citrates* and *Imperata cylindrical*. The higher amount of phenols are important in the regulation of plant growth, development and disease resistance. It can be used as fungicide, pesticides, antiseptic and disinfectant and in the manufacture of resins. Plant phenols may interfere with stages of Cancer process. Potentially resulting in a reduction of cancer risks.<sup>34</sup> Over the last few years, several experimental studies have revealed biological and pharmacological properties of phenolics compounds, especially their anti-inflammatory activity, antiviral and cytotoxic activity.<sup>35</sup> Phenolics are active in curing kidney and stomach problems as well as helpful as anti-inflammatory in action.<sup>36</sup> Phenols are reported antitumour agents and to exhibit antiviral and antimicrobial activities,<sup>37</sup> hypotensive effects and antioxidant properties.<sup>38</sup> Phenols are said to offer resistance to diseases and pest in plants.<sup>39</sup>

**Figure 1:** Quantification analysis of alkaloid content from grasses of cyperaceae**Figure 2:** Quantification analysis of flavonoid content from grasses of cyperaceae

Quinones are present in five plants of selected species. Quinones are known to act as mobile electron carriers within the lipid phase of the membrane in photosynthetic and respiratory electron transport chains.<sup>40</sup> The function of quinones is essential for proton-based energy coupling and specific binding sites, allowing only two-electron transfer steps, prevent damage of the cell by free-radical formation.<sup>41</sup>

Saponin content are present in maximum number of selected species showed. Maximum levels of saponins

were present in *Cyperus rotundus* 0.318 and lower level in *Kyllinga monoceps* 0.008 mg/g dw. Traditionally saponins have been extensively used as detergents as pesticides and molluscicides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects.<sup>11</sup> Saponins has relationship with sex hormones like oxytocin, which is a sex hormone involved in controlling the onset of labour pains in female and the subsequent release of milk.<sup>42</sup> Saponins enhance nutrient absorption and aid in animal digestion. They are bitter to taste and so, can reduce plant palatability or even imbue them with life threatening animal toxicity.<sup>43</sup> There is tremendous, commercially driven promotion of saponins in traditional medicine preparation,<sup>44</sup> where oral administration might be expected to lead the hydrolysis of glycosides from terpenoid and obviation of many toxicity associated with the intact molecule. Saponin protects the plant against microbes fungi and viral diseases.<sup>45</sup> Reported that, saponins had expectorant action which is very useful in the management of upper respiratory tract inflammation and Saponins have been found to possess hypocholesterolemic property for the control of high blood lipids.<sup>46-47</sup> Steroids are present in *Cyperus rotundus*, *Fimbristylis monostachya*, *Kyllinga monoceps*, *Kyllinga triceps* and *Scelaria lithosperma*. Steroids are present in grass *Cyperus rotundus*,<sup>25</sup> *Cymbopogon citratus*,<sup>29</sup> *Cynodon dactylon* and *Sacchrum spontaneum*.<sup>22-26</sup> It should be noted that steroidal compounds are of importance and of interest in pharmacy due to their relationship with sex hormones.<sup>48</sup>

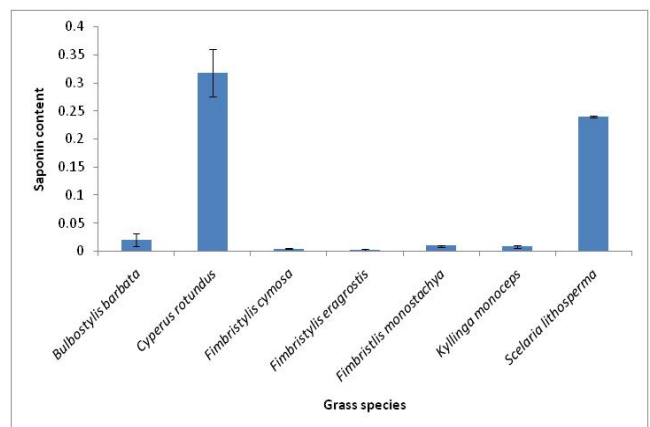


**Figure 3:** Quantification analysis of phenol content from grasses of cyperaceae

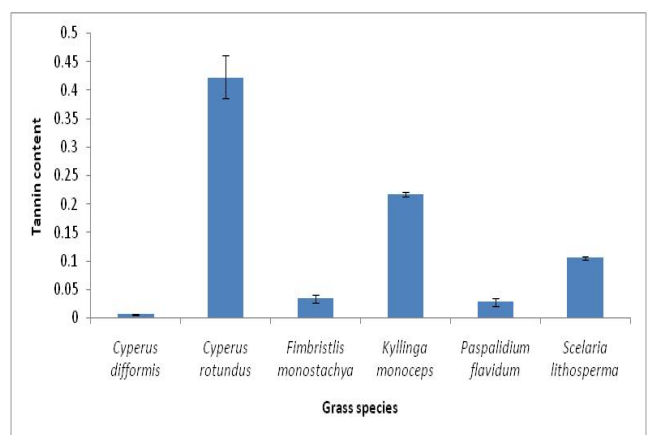
Tannins are present in six plants among selected species. Maximum content found in *Cyperus rotundus* followed by *Kyllinga monoceps*, which are also reported in *Cyperus rotundus*,<sup>25</sup> *Cymbopogon citratus* by,<sup>21-29</sup> *Sacchrum spontaneum* by,<sup>26</sup> *Imperata cylindrical* and *Pennicetum purpureum*.<sup>27-28</sup> Tannins contribute property of astringency i.e. fasten the healing of wound and inflamed mucous membrane and have received considerable attention in the fields of nutrition, health and medicine, largely due to their physiological activity, such as antioxidant, antimicrobial and anti-inflammatory

properties. Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery.<sup>49</sup> Tannins and tannic acid owe their stringent action to the fact that they precipitate protein and render them resistant to attack by proteolytic enzymes, internally they form a pellicle of coagulated protein over the lining of the alimentary tract.

Terpenoids are present in *Cyperus difformis* and *Kyllinga monoceps*. Terpenoids which are attributed for analgesic and anti-inflammatory activities. The results revealed that *Cyperus rotundus* may be the best source for anti-microbial, anti-carcinogenic, anti-inflammatory and increase in milk production among twenty selected species as it is rich in phenols and saponins.



**Figure 4:** Quantification analysis of saponin content from grasses of cyperaceae



**Figure 5:** Quantification analysis of tannin content from grasses of cyperaceae

## CONCLUSION

The species of cyperaceae screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the animals as a result of the presence of various compounds that are vital for good health. These can be incorporated in other foods as nutraceuticals for effective and proper metabolism as well as for the maintenance of good physiological state in man and animals. The study has revealed that the *Cyperus*

*rotundus*, *Kyllinga monoceps*, *Paspalidium flavidum* and *Scelaria lithosperma* grass species possess certain medicinal values due to their phytochemical contents which can be utilized in the treatment of many diseases and also be explored for use in pharmaceutical industries, food preparation, and also in areas like hair and skin, cosmetics and raw materials for other products. From the above studies, it is concluded that the grass species possess medicinal values represents new sources of pharmaceutical industries with stable, pharmacologically active secondary metabolites which can be used as healers in the field of medicine. Flavonoids, alkaloids and steroids were found to be present as active constituents in the plant extracts. These extracts can be used for the treatment of many infectious diseases like diarrhea, dysentery, typhoid, pneumonia and other pathogenic disorders. However, further research is required to establish the *in vivo* activities and therapeutic index of these plants with respect to the management of various pathogenic diseases and improve the health of livestock.

**Acknowledgement:** Authors are grateful to UGC for financial assistance.

## REFERENCES

- Ahmed F, Urooj A, Journal of young pharm, 2, 2010, 160-164.
- Zheng W, Wang SY, Journal of Agricultural Food Chemistry, 49, 2001, 5165–5170.
- Thitilertdech N, Teerawutgulrag A, Rakariyatham N, Antioxidant and antibacterial activities of *Nephelium lappaceum* L. extracts. Food Science and Technology, 41, 2008, 2029-2035.
- Sengul, M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S, Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants, Pakistan Journal of Pharmaceutical Sciences, 22(1), 2009, 102-106.
- Rout SP, Choudhary KA, Kar DM, Das L, Jain A, Plants in traditional medicinal system- future source of new drugs, Internal J Pharmacy & Pharmaceutical Sci., 1(1), 2009, 1-23.
- Anyasor GN, Ogunwenmo KO, Oyelana OA, Akpofunure BE, Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl. (Costaceae), African Journal of Biotechnology, 9(31), 2010, 4880-4884.
- Igbinosa OO, Igbinosa EO, Aiyegoro OA, Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn), African Journal of Pharmacy and Pharmacology, 3(2), 2009, 058-062.
- Havsteen B, Flavonoids, A class of natural products of higher pharmacological potency, Biochemical Pharmacology, 32, 1983, 1114-1148.
- Middleton E, The flavonoids, Trends in Pharmaceutical Science, 5, 1984, 335-338.
- Kim SY, Kim JH, Kim SK, Ohhandy MJ, Yung MY, Antioxidant activities of selected oriental herb extracts, J Am Oil Chem Soc., 71, 1984, 633-640.
- Okwu DE, Phytochemicals and vitamin content in indigenous spices of South Eastern Nigeria, J Subs Agric, Environ., 6, 2004, 30-34.
- Barile E, Bonanomi G, Antignani V, Zolfaghari B, Ebrahim Sajjadi S, Scala F, Lanzotti V, Phytochemistry, 68, 2007, 596-603.
- Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO, Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria, Trop J Pharm Res., 7, 2008, 1019-1024.
- Mohanta, TK, Patra JK, Rath SK, Pal DK, Thatoi HN, Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semecarpus anacardium* L. Scientific Research and Essay, 2(11), 2007, 486-490.
- Harbone JB, Phytochemicals methods, London, Chapman and Hill, 1973.
- Gibbs RD, Chemotaxonomy of Flowering Plants, McGill Queen's University Press, Montreal and London, 1, 1974.
- Peach K, Tracey MV, Modern methods of plant analysis, Springer Verlag, Berlin, 3, 1956.
- Cheng KT, Wong TY, Wei CL, Huang YW, Lin Y, Tannins and human health, A review Criti Rev. Food Sci Nutri., 6, 1998, 421-464.
- Treare GE, Evans WC, Pharmacognosy, Bahive Tinal, London, 17<sup>th</sup> Ed, 1985, 149.
- Makkar HPS, Blummel M, Borowy NK, Becker K, Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods, J Sci Food Agri., 61, 1993, 161-165.
- Asaolu MF, Oyeyemi OA, Olanlokun JO, Chemical Compositions Phytochemical Constituents and *in vitro* Biological Activity of Various Extracts of *Cymbopogon citrates*, Pakistan Journal of Nutrition, 2009, 1920-1922.
- Jarald EE, Joshi SB, Jain DC, Antidiabetic activity of aqueous extract and non polysaccharide fraction of *Cynodon dactylon* pers, Indian Journal of Experimental Biology, 46, 2008, 660 -667.
- Harborne JB, Phytochemical methods, Chapman and Hall publications, London, Newyork, 2nd edition, 1984, 288.
- Liu H, Extraction and Isolation of Compounds from Herbal Medicines. In, Willow, JH, Liu (Eds.) Traditional Herbal Medicine Research Methods, John Wiley and Sons, Inc, 2011.
- Ranjani S, Jeyadevan P, Physico-Chemical and Phyto-Chemical Study of Rhizome of *Cyperus rotundus* Linn, International Journal of Pharmacology and Pharmaceutical Technology (IJPPT), 1(2), 2012, 2277-3436.
- Khalid M, Hefazat H, Siddiqui, Pharmacognostical Evaluation and Qualitative Analysis of *Saccharum spontaneum* (L.) Root, International Journal of Pharmaceutical Sciences and Drug Research, 3(4), 2011, 338-341.
- Padma R, Parvathy NG, Renjith V, Kalpana P Ronate, Quantitative estimation of tannins, phenols and antioxidant activity of methanolic extract of *Imperata cylindrical*, Int J Res Pharm Sci., 4(1), 2013, 73-77.



28. Okaraonye CC, Ikwuchi JC, Nutritional and Antinutritional Components of *Pennisetum purpureum* (Schumach), Pakistan Journal of Nutrition, 8(1), 2009, 32-34.
29. Omotade I, Oloyede, Chemical profile and antimicrobial activity of *Cymbopogon citratus* leaves, Journal of Natural Products, 2, 2009, 98-103.
30. Linuma M, Tsuchiya H, Sato M, Yokoyama J, Ohyama M, Ohkawa Y, Tanaka T, Fujiwara S, Fujii T, Flavanones with potent antibacterial activity against methicillin-resistant *Staphylococcus aureus*, J Pharmacol., 46(11), 1994, 892-895.
31. Salah W, Miller N, Pagauga G, Tybury G, Bolwell E, Rice E, Evans C, Polyphenolic flavonoids as scavenger of aqueous phase radicals and chain breaking antioxidants, Arch. Biochem., 2, 1995, 239-346.
32. Balch JF, Balch PA, Prescription for Nutritional Healing, New York, A very, Penguin Putnam Inc., 2000, 267-270.
33. Cambell MM, Sederoff RR, Variation in lignin content and composition, 100, 1996, 3-13.
34. Hollman PCH, Evidence for health effects of plant phenols local or systemic effects, J Sci Food Agri., 81, 2001, 842-852.
35. Shirwaikar A, Malini S, Kumari SC, Protective effect of *Pongamia pinnata* flowers against cisplatin and gentamicin induced nephrotoxicity in rats, Indian J Exp Biol., 1, 2003, 58-62.
36. Zhu M, Phillipson D, Greengrass PM, Bowery NE, Cai Y, Plant polyphenols: biologically active compounds or non-selective binder to protein? Phytochemistry, 44(3), 1997, 441-447.
37. Robbins R, Medical and nutritional aspects of citrus bioflavonoids, In Nagy, S and J Attaway, (Eds), Citrus nutrition and quality. American chemistry society, Washington, DC, 43-59.
38. Robak J, Gryglewski RJ, Flavonoids are scavengers of superoxide anions, Biochem Pharmacol, 37, 1988, 837-841.
39. Sadasivam S, Manickam AC, Biochemical methods for agricultural sciences – Phenolics, Publishing for one world, Wiley Eastern Limited and TamilNadu Agricultural University, Coimbatore, 1991, 187-188.
40. Fato R, Estornell E, Di Bernardo S, Pallotti F, Parenti Castelli G, Lenaz G, Steady-state kinetics of the reduction of coenzyme Q analogs by complex I (NADH: ubiquinone oxidoreductase) in bovine heart mitochondria and submitochondrial particles, Biochemistry, 35, 1996, 2705-2715.
41. Barr R, Pan RS, Crane FL, Brightman AO, Morre DJ, Destruction of vitamin K1 of cultured carrot cells by ultraviolet radiation and its effect on plasma membrane electron transport reactions, Biochem Int, 27, 1992, 449-456.
42. Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y, Saponins from edible legumes, Chemistry, processing and health benefits, J Med Food, 7, 2004, 67-78.
43. Forester H, "Meta eye pathway: Saponin biosynthesis", Retrieved from, 2006.  
(<http://www.answer.com/topic/saponin>) on 5/6/2009.
44. Asl MN, Hossein Z, Review of pharmacological effects of *Glycyrrhiza sp.* and its bioactive compounds, Phytotherapy Research., 22(6), 2008, 706 – 724.
45. Shahidulalam M, Quader MA, Rashid MA, HIV inhibitory diterpenoid from *Anisomeles indica*. Fito., 71, 2000, 574-576.
46. Finar IL, Organic chemistry, Stereochemistry and the chemistry of natural products, 5th edition, Volume 2, Longman group, U.K, 198, 517-605.
47. Oakenfull D, Sidhu GS, Could Saponins be a useful treatment for hypocholesterolemia, European Journal of Clinical Nutrition, 44, 1990, 79-88.
48. Santhi R, Lakshmi G, Priyadarshini AM, Anandaraj L, Phytochemical screening of *Nerium oleander* leaves and *Momordica charantia* leaves, Inter Res J Pharm., 2, 2011, 131-135.
49. Dharmananda S, Gallnuts and the Uses of Tannins in Chinese Medicine –A paper delivered at Institute for Traditional Medicine, Portland, Oregon 2003.

Source of Support: Nil, Conflict of Interest: None.