Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(2); 240-245

ISSN: 0975-4873

Research Article

Evaluation of HPTLC Fingerprints of Flavonoids and Antioxidant Activity of Selected Medicinal Plants of Lamiaceae Family

Asha.D[1*], Lizzy Mathew[1], Rishad. K.S.[2]

[1]*Department of Botany, St.Teresa's College, Ernakulam, Kerala, India.*
[2]*School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India.*

Available Online: 1st March, 2015

ABSTRACT

The main objective of the present study was to evaluate the phytochemical constitution, antioxidant activity and flavonoid profiling of methanolic extracts of aerial parts of four important herbs of Lamiaceae family *Ocimum basilicum* L.., *Mentha arvensis* L., *Hyptis suaveolens* L.(Poit.) *and Coleus aromaticus* Benth.. The preliminary phytochemical screening revealed the presence of Tannins, Saponins, Flavonoids, Triterpenoids, Steroids, Cardiac glycosides and Alkaloids. Antioxidant activity of plant extracts were determined by quantitative analysis of phenol & DPPH radical scavenging assay. The flavonoid profiles of these plants were also studied using HPTLC and data were analysed using WinCATS 4 software. The estimation of phenol showed the maximum in *Coleus aromaticus* Benth. (36mg/g) followed by *Mentha arvensis* L. *and Hyptis suaveolens* L.(Poit.) (24mg/g). The highest value for DPPH assay was recorded in *Mentha arvensis* L.(37.01%) followed by *Hyptis suaveolens* L.(Poit.) (35.76%) while comparatively lower amount (18.17%) with *Ocimum basilicum* L. Comparative study of the extracts, successfully demonstrated significant variation of different phenolic compounds and also non enzymatic antioxidant activity in the four plant species . The fingerprint profile generated by HPTLC could be useful for future reference in discriminating the plant species and also detecting adulteration and substitution of raw drugs in Indian systems of medicine.

Key words: Medicinal plants, Phytochemical screening, HPTLC finger printing, Antioxidant activity

INTRODUCTION

The medicinal plants are important therapeutic aids for curing various ailments since ancient times. In the recent past, there has been a tremendous increase in the use of plant based health products in developing as well as developed countries because of their safety aspects [1]. A large number of medicinal plants are now widely used all over the world for production of both traditional and modern drugs and development of new drugs. But Indian herbal drugs have still low acceptability in the world market due to insufficient scientific validation. The plant kingdom constitutes most widely distributed extremely heterogeneous groups of the substances called as PSMs (Plant Secondary Metabolites) [2]. In various *in vitro* assay studies, PSMs present in various plants and their extracts reported as excellent radical scavenger and thereby exhibit therapeutic property [3, 4]. However, in order to make sure the safe use of these medicines, it is necessary to establish the standards of quality, safety and efficacy of the drug. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations[5]. Lamiaceae, also called Labiatae, the mint family of flowering plants, with 236 genera and more than 7,000 species, the largest family of the order Lamiales. The plants are aromatic and very important to humans in having flavour, fragrance and medicinal properties. These herbs are used in traditional medicine, but are mainly known for their culinary properties. Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants and occur in many Lamiaceae members. High Performance Thin Layer Chromatography (HPTLC) is a sophisticated, reliable, efficient and automated form of TLC having the latest technical developments for quality assessment and evaluation of botanical materials[6]. A chromatographic fingerprint of extract represents a chromatographic pattern of pharmacologically active or chemically characteristic constituents present in the extract[7, 8]. So, the present study was designed to determine the phenolic compounds using HPTLC profile of the important plants of the Lamiaceae family, *Ocimum basilicum* L.., *Mentha arvensis* L., *Hyptis suaveolens* L.(Poit.) *and Coleus aromaticus* Benth. Keeping in view the importance of the these plants as spices as well as medicines, the present investigation was carried out as preliminary screening of the secondary metabolites, to establish their HPTLC profiles and

**Author for Correspondence*

Table 1: Preliminary Phytochemical Screening of the selected plants

S.No.	Test	*Ocimum basilicum*	*Hyptis suaveolens*	*Mentha arvensis*	*Coleus aromaticus*
1.	Tannin	+	+	+	+
2.	Saponin	+	+	+	+
3.	Flavonoids	+	+	+	+
4.	Steroids	+	+	+	+
5.	Triterpenoids	+	+	+	+
6.	Cardiac glycosides	+	+	-	+
7.	Alkaloids	+	+	+	+

(+ = Present, - = Absent)

Table 2: HPTLC - Flavonoids profile of the methanolic extracts of *Hyptis suaveolens* L.(Poit.)

Flavonoids	Rf	Peak Height	Area%
Gallic acid	0.09	104.906	11.03
Ferulic acid	0.25	225.271	76.90
Quercetin	0.43	37.630	8.94
Chlorogenic acid	0.63	23.990	2.42
Rutin	0.84	97.61	0.71

Table 3: HPTLC - Flavonoids profile of the methanolic extracts of *Mentha arvensis* L.

Flavonoids	Rf	Peak Height	Area%
Gallic acid	0.09	16.752	1.50
Ferulic acid	0.25	316.296	84.26
Quercetin	0.43	50.071	6.49
Chlorogenic acid	0.63	47.593	7.76
Rutin	Not Detected	Not Detected	Not Detected

Table 4: HPTLC - Flavonoids profile of the methanolic extracts of *Coleus aromaticus* Benth.

Flavonoids	Rf	Peak Height	Area%
Gallic acid	0.09	91.68	5.51
Ferulic acid	0.25	448.937	78.49
Quercetin	0.43	119.887	14.78
Chlorogenic acid	0.63	13.780	0.61
Rutin	0.84	13.780	0.61

Table 5: HPTLC - Flavonoids profile of the methanolic extracts of *Ocimum basilicum* L.

Flavonoids	Rf	Peak Height	Area%
Gallic acid	0.09	117.19	12.89
Ferulic acid	0.25	404.418	61.16
Quercetin	0.43	54.045	8.46
Chlorogenic acid	0.63	20.054	2.73
Rutin	0.84	59.001	14.75

antioxidant activity which would help in the authentication of the plants and also to elucidate bioactive compounds present in the plant system.

MATERIALS AND METHODS

Fresh aerial parts of the selected plants were collected from the Ernakulam district, Kerala and analyzed for qualitative and quantitative phytochemical evaluation. The collected plants were washed in running water for several times followed by distilled water and shade dried at room temperature for 30 days. Dried specimens were powdered using the electric homogenizer and stored in airtight plastic covers in low temperature for further phytochemical investigations.

Preparation of the extract

About 20 gm of the powdered sample was extracted with 200 ml hot methanol in a soxhlet apparatus. The extract is then concentrated in a boiling water bath to a volume of 30 ml and stored in air tight small brown bottles. These extracts were subjected to phytochemical screening for the identification of various phytoconstituents.

Preliminary phytochemical screening

The preliminary phytochemical screening with various qualitative chemical tests were performed to detect the

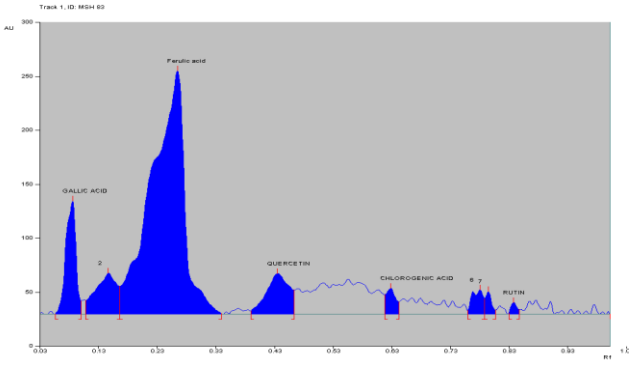

Fig1: HPTLC Chromatogram of *Hyptis suaveolens* L. (Poit.)

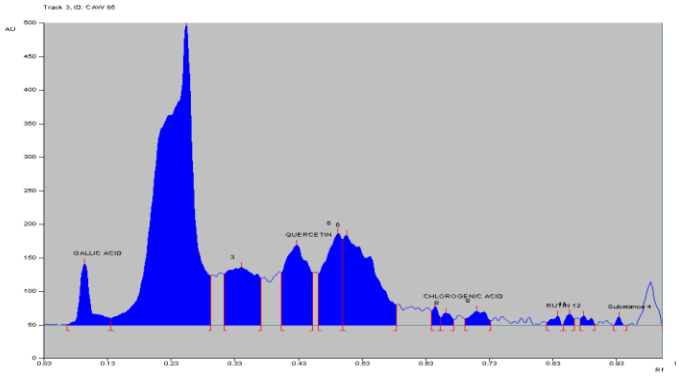

Fig 3: HPTLC Chromatogram of *Coleus aromaticus* Benth.

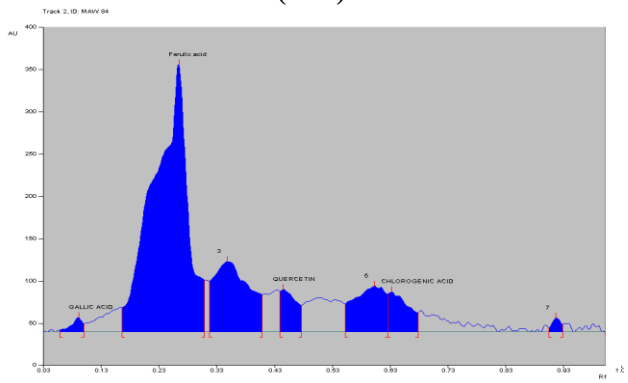

Fig 2: HPTLC Chromatogram of *Mentha arvensis* L.

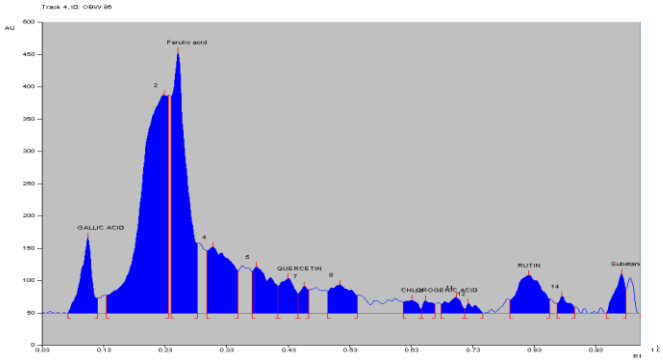

Fig 4: HPTLC Chromatogram of *Ocimum basilicum* L.

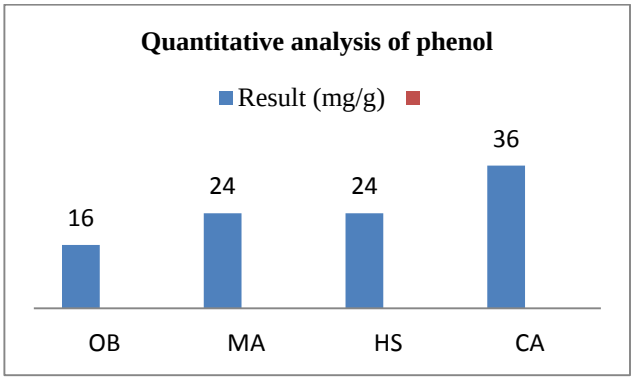

Fig: 5: Representing the quantitative determination of Phenol in *Ocimum basilicum* L., *Mentha arvensis* L., *Hyptis suaveolens* L.(Poit.) and *Coleus aromaticus* Benth.
(Note: OB= *Ocimum basilicum* L., MA = *Mentha arvensis* L., HS = *Hyptis suaveolens* L.(Poit.)., CA= *Coleus aromaticus* Benth.)

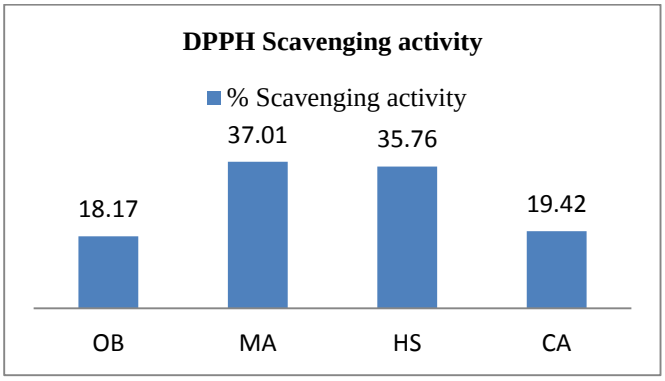

Fig: 6: Representing the DPPH Scavenging activity in *Ocimum basilicum* L., *Mentha arvensis* L., *Hyptis suaveolens* L.(Poit.) and *Coleus aromaticus* Benth.
(Note: OB= *Ocimum basilicum* L., MA = *Mentha arvensis* L., HS = *Hyptis suaveolens* L.(Poit.)., CA= *Coleus aromaticus* Benth.)

presence of various classes of phytoconstituents like alkaloids, glycosides, flavonoids, phenols and tannins, saponins, sterols and triterpenoids. These phytoconstituents were identified by characteristic color changes using standard procedures [9, 10, 11].

HPTLC Fingerprinting of Extract

HPTLC studies were carried out following the standard methods [12]. Methanol extract of the selected plants (10mg/ml) and collected fractions residue (1mg/ml) was subjected to HPTLC (CAMAG, Switzerland) analysis. Extract and each fraction were spotted on a silica gel 60F254 (Merck, Germany) TLC plate. The plate was air dried and then developed by using the solvent system Ethyl acetate-Acetic acid-Formic acid-Water 100:11:11:27 (v/v/v/v) as mobile phase in a CAMAG- twin-trough glass chamber (20x10x4) previously saturated with mobile phase vapor for

20 min. After developing the plate, it was dried at 105ºC for 15 minutes and then it was scanned using Scanner 3 (CAMAG, Switzerland) at 366 nm using WinCATS 4 software. Chromatograms were evaluated before and after spraying with 5% NP-PEG reagent in Alcohol.

Screening Antioxidant Activity

All plant extracts were subjected for the screening antioxidant activity by implementing standardized protocols. The chemicals utilized were of pure and analytical grade.

Phenol estimation by Folin-Ciocalteau method[13]

To 0.5 ml of extract 2.5 ml of Folin–Ciocalteau reagent (diluted 10 times with water) was added and, after 3 minutes 2 ml of sodium carbonate (Na2CO3) (75 g/L) was added and the sample was mixed. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at room temperature, the absorbance was measured at 765 nm using a spectrophotometer. Total phenols were expressed as gallic acid equivalents (GAE)/100 g dry weight (DW) of the sample. The concentration of total phenolics is expressed as milligram of gallic acid /g of sample.

DPPH radical scavenging assay[14]

20µl (1µg/10µL) of the extracts were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the leaf extracts, served as the positive control. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518 nm in a spectrophotometer [15, 16]. The radical scavenging activity was calculated as follows:

$$\%DPPH\ Radical\ Scavenging\ Actity\ (RSA) = \frac{A518(sample) - A518(blank)}{518(blank)} \times 100$$

RESULTS AND DISCUSSIONS

Phytochemical screening on methanolic extracts of *Ocimum basilicum* L.., *Mentha arvensis* L., *Hyptis suaveolens* L.(Poit.) *and Coleus aromaticus* Benth. revealed the presence of Alkaloids, Saponins, Tannins, Triterpenoids, Steroids, Cardiac glycosides and Flavonoids (Table 1).

Various solvent compositions of the mobile phase for HPTLC analysis were examined in order to achieve high resolution and reproducible peaks. The mobile phase with the composition of Ethyl acetate-Acetic acid-Formic acid-Water (100:11:11:27) showed high resolution and repeated results confirmed their efficiency and accuracy. The methanolic extract of the four plants illustrated the presence of different types of phenolic compounds with different Rf values. The chromatograms showed spots which are characteristic for several Lamiaceae species[17]. More than 10 peaks were found in all the four tracks indicating the presence of 10 different compounds in the methanolic extract of the selected plants. Spots with Rf 0.09,0.25, 0.43, 0.63 and 0.84 (Fig 1-4) were found to be common in all the extracts indicating the presence of Gallic acid, Ferulic acid, Quercetin ,Chlorogenic acid and Rutin respectively which appeared as intense fluorescent spots on tracks corresponding to the particular plants.

In *Hyptis suaveolens, Mentha arvensis, Coleus aromaticus and Ocimum basilicum*, out of 10 components, the component Ferulic acid with Rf value 0.25 was found to be prominent as the percentage area was more with 76.90%, 84.26%, 78.49% and 61.16% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 14% (Table 2-5). HPTLC fingerprinting is proved to be a reliable, accurate and precised method for herbal identification and authentication. Thus the developed chromatogram and Rf value will be specific with selected solvent system, and serve the better tool for standardization of the extracts of the selected lamiaceae plants. Most importantly, this study can be used to isolate and characterize the chemical constituents present in these plant extracts.

Antioxidant Activity

Determination of Total Phenolics

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy metal chelators and hydroxyl radical quenchers [18]. The highest % phenol recorded among the studied medicinal plants was in *Coleus aromaticus* Benth. followed by *Mentha arvensis* L. *and Hyptis suaveolens* L.(Poit.). While comparatively lower % phenol obtained with the methanol extracts of *Ocimum basilicum* L. (Fig-5). Measurement of the polyphenols and free radical scavenging activity of herbs has become important tools for the understanding of the relative importance of plant species especially from the health point of view[19].

Free radical scavenging assay

The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The methanol extract of *Ocimum basilicum* L. , *Mentha arvensis* L. , *Hyptis suaveolens* L.(Poit.) and *Coleus aromaticus* Benth. exhibited an efficient (18.17%, 37.01%, 35.76%, 19.42% respectively) inhibition of DPPH free radical (Fig- 6). The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports. It was reported that a fair correlation is between antioxidant and free radical scavenging activity and phenolic contents[20]. Phenolic antioxidants present in herbs have the ability to reduce lipid peroxidation, prevent DNA oxidative damage and scavenge ROS (Reactive oxygen species) like superoxide, hydrogen peroxide and hydroxyl radicals[21]. DPPH assay confirmed the good antioxidant potential of the Lamiaceae species. The most efficient activity was exhibited by *Mentha arvensis* and *Hyptis suaveolens*. In our study this fact proves that the antioxidant activity is given not only by Phenolic compounds but also due to the contribution of other compounds, like volatile oils, tri- or diterpenes. It has been reported that the antioxidant activity of many compounds of botanical origin is proportional to antioxidant content

suggesting a correlation between total phenolics and antioxidant activity [22, 23]. The study on the correlation between free radical scavenging and total phenol content of eight Lamiaceae plants found in Manipur showed 51% of the free radical scavenging is contributed by phenolic compounds[24]. Thus it is clear that polyphenolic compounds in the selected plants play an important role as bioactive principles and the scavenging effect can be attributed to the presence of active phytoconstituents in them. Moreover, the phyto-chemical profile of the plant extracts helps in understanding the extent of antioxidant activity[25].The finding of this study suggests that these plants could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing many diseases.

CONCLUSION

Phytoconstituents are the natural bioactive compounds found in plants. The medicinal plants contain many of the phytoconstituents in which the phenolic compounds, could be used for many therapeutic purposes as they often exhibit a huge amount of medicinal properties such as antioxidant, anticarcinogenic, antitumor, antidiabetic, anti-inflammatory activities. The results in this work demonstrate that the extracts of the selected medicinal plants of the Lamiaceae family contain a considerable amount of phenols, flavonoids and antioxidant potentials. A comparative HPTLC analysis of different plant extracts represents comparative account of the amount of flavonoids present in this particular family. Further investigation on the isolation, purification and characterization of the phytochemical constituents in these plants may reveal the potentiality of these compounds in pharmaceutical industry.

ACKNOWLEDGEMENT

The authors are grateful for the support of St.Teresa's College, Ernakulam, Kerala and UniBiosys Biotech Research labs, Kalamassery, Ernakulam, Kerala for providing facility to carry out this Research.

REFERENCES

1. Panday MM, Rastogi S, Rawat AKS. Indian herbal drug for general health care on overview. The international journal of alternative medicine 2008 ; 6: 1.
2. Harborne JB . A Guide to Modern Techniques of Plant Analysis. In: Phytochemical Methods edited by Harborne JB. 3rd edition, Chapman and Hall, Hong Kong, 1984.
3. Patel RM, Jasrai YT . Antioxidant activity of medicinal spices and aromatic herbs. Annals of Phytomedicine 2012; 1(1): 75-80.
4. Nahar L, Ripa FA, Rokonuzzaman, Alim Al- Bari MA. Investigation on antioxidant activities of six indigenous plants of Bangladesh. Journal of Applied Sciences Research 2009; 5(12): 2285-2288.
5. Chaudhay Ranjit R. Herbal Medicine for Human Health. Regional Publication, SEARO, No. 20, W.T.O, New Delhi, 1992, 1-80.
6. Saraswathy A, Shakila R, Sunilkumar KN . HPTLC fingerprint profile of some *Cinnamomum* species. Pharmacognosy Journal 2010; 2: 211-215.
7. Bhise SB, Salunkhe VR . Formulation of health drinks using natural sweetener, its HPTLC method development and validation. Journal of Pharmacognosy and Phytotherapy 2009; 1: 014-020.
8. Sanja SD, Sheth NR, Patel NK, Patel D, Patel B. Characterization and evaluation of antioxidant activity of *Portulaca oleracea*. International Journal of Pharmacy and Pharmaceutical Sciences 2009; 1: 74-84.
9. Khandelwal K., "Practical Pharmacognosy", Nirali Publication, 2nd edition, p. 183–184.
10. Harborne JB. Phytochemical Methods. Chapman and hall Ltd.,London: U.K., 1973, 49-188.
11. Thiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review.Internationale Pharmaceutica Scientia 2011; 1(1):92-106.
12. Savithramma N, Linga M, Suhrulatha D. Screening of medicinal plants for secondary metabolites. Middle East Journal of Scientific Research 2011; 8(3):579-584.
13. Yadav RNS, Agarwal M. Phytochemical analysis of some medicinal plants. Journal of Phytology 2011;3(12):10-14.
14. Singleton VL, Rossi JA Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am.J.Enol. Vitic 1965; 16:144-158.
15. Brand- Williams W, Cuvelier ME and Berset C. Use of free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft and Technologie 1995; 28(1):25-30.
16. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA . Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pakistan Journal of Pharmaceutical Sciences 2009; 22(3): 277-81.
17. Aquil F, Ahmad I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turkish Journal of Biology 2006; 30: 177-183.
18. Armatu A, Colceru-mihul S, Bubueanu C, Draghici E, Piravu L. Evaluation of antioxidant and free scavenging potential of some Lamiaceae species growing in Romania. Romanian Biotechnological Letters 2010; 15:.3.
19. Patel RM and Jasrai YT . Plant secondary metabolites and their commercial production. South Asian Journal of Social and Political Sciences 2009; 9(2): 115-122.
20. Chang WC, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, Park SH, Kim SK. Antioxidant activity and

free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci 2002;163: 1161-1168.

21. Singh R, Singh B, Singh S, Kumar N, Kumar S, Arora S . Investigation of ethyl acetate extract/fractions of *Acacia nilotica* willd. Ex Del as potent antioxidant. Records of Natural Products 2009; *3*(3): 131-138.
22. Yoo KM, Lee CH, Lee H, Moon BK, Lee CY. Relative antioxidant and cytoprotective activities of common herbs. Food Chemistry 2008; 106: 929-936.
23. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997; 2: 152-159.
24. Veglioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 1998; 46: 4113-4117.
25. Sandhyarani D Khomdram, Potsangbam K Singh. Polyphenolic Compounds and Free Radical Scavenging Activity in Eight *Lamiaceae* Herbs of Manipur. Not Sci Biol. 2011;3(2):108-113.
26. Riddi M Patel. Comparative Antioxidant activity evaluation and HPTLC phyto-chemical fingerprinting of some Indian medicinal plant extracts. Cibtech Journal of Bio-Protocols 2014; 3(1): 19-34.