IJPSR (2015), Vol. 6, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 August, 2014; received in revised form, 31 October, 2014; accepted, 19 December, 2014; published 01 April, 2015

A COMPARITIVE STUDY OF PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY AMONG TRADITIONAL MEDICINAL PLANTS

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Keywords:

Phytochemicals, Antioxidant activity, Lipid peroxidation, Reducing power activity, *Sida cardifolia*, *Phyllanthus amarus* and *Adathoda vasica*

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ABSTRACT: Acetone extracts of the three medicinal plants *Sida cardifolia, Phyllanthus amarus* and *Adathoda vasica* were assessed for phytochemical components qualitatively and quantitatively and antioxidant activity. The results revealed that all the three contained alkaloids, carbohydrates, flavonoids, tannins, anthocyanins, steroids and phenols. Saponins were absent in all the three extracts. *Sida cardifolia* with 1.53mg and 7.09mg GA equivalent/g of extract powder showed maximum amount of Phenolics and flavonoids.The total sugars and proanthocyanidins was maximum in *Phyllanthus amarus* with 8.45ug glucose equivalent/g of extract powder and 10.84mg GA equivalent/g of extract powder. Tannin content was found to be high in *Adathoda vasica* with 11.07mg GA equivalent/g of extract powder. Sida cardifolia acetone leaf extract showed the highest reducing power activity, Free radical scavenging potential and inhibition of lipid peroxidation.

INTRODUCTION: In a healthy body prooxidants and antioxidants maintain a ratio and a shift in this ratio towards prooxidants gives rise to oxidative stress. This oxidative stress may be either mild or severe depending on the extent of shift. This causes several diseases such as cardiovascular diseases, neurological diseases, renal diseases, diabetes, skin diseases, ageing, respiratory diseases and inflammatory problems [1, 2]. Over 70 degenerative diseases are due to oxidative stress. These free radicals are produced from two important sources, cellular metabolism and environmental sources.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(4).1609-15

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(4).1609-15

Free radicals are also beneficial components of inflammatory response and hepatic cytP450 mediated detoxification. These free radicals generated at the site of inflammation serves as a part of defensive mechanism by harming the invading pathogens. The generation of free radicals persisted even after the defensive role results in the more deleterious complications. In such conditions regulation of these free radicals is equally important. A lot of researches are going on worldwide directed towards finding natural antioxidants of plants origins. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities [3, 4, 5].

In India, the use of different parts of several medicinal plants to cure specific ailments has been in vague from ancient times. Therefore, now there

is a need to look back towards the traditional medicine which can serve as novel therapeutic agent. Active research has been driven in recent years on plant components due to their biologically beneficial effects emanating from antioxidant activities of phenolic phytochemicals. The antioxidant activity is mainly due to the presence of phytochemicals such as phenolics, flavonoids and anthocyanins.

The aim of this study was to evaluate the phytochemicals qualitatively and quantitatively and analysis of *in vitro* antioxidant activities of acetone extracts of the three medicinal plants *Sida cardifolia, Phyllanthus amarus* and *Adathoda vasica.* The entire plant of *Sida cardifolia* is used in making traditional medicine for inflammations, bleeding disorders, fevers, delirium and urinary problems. *Phyllanthus amarus* has also shown to work as an antifungal, antibacterial, antiviral agent, stomachic, diuretic, febrifuge and antiseptic. *Adathoda vasica* leaves have been used extensively in Ayurvedic Medicine for over 2000 years primarily for respiratory disorders. The leaves are boiled and combined with honey, ginger and black pepper to treat coughs and respiratory ailments.

MATERIALS AND METHODS:

Collection of plant material:

The leaves of *Sida cardifolia, Phyllanthus amarus and Adathoda vasica* were collected from local places of Mysore. The leaves were separated manually, dried, powdered and stored in airtight containers until further studies.

Preparation of Extract: 10 g of each powder was extracted in 20ml of acetone by stirring using a magnetic stirrer at cold condition for 4 hours. The extracts were centrifuged at 10,000rpm for 10 minutes and then filtered through Whatman no.1 filter paper for removal of particulates. The residues obtained were re extracted with another 20ml of acetone and again the process was repeated. The acetone extracts of different parts were pooled separately and concentrated under vaccum at 40°C. The yield was calculated and expressed as percentage of w/w.

Preliminary phytochemical screening: The qualitative analysis of acetone extracts for the presence of various phytochemicals was carried out using the standard procedures with little modifications [6, 7].

TABLE: 1 PERCENTAGE YIELD OF EXTRACTS

Sl No	Acetone Extracts	Yield (%W/W)
1	*Sida cardifolia*	2.82
2	*Phyllanthus amarus*	4.09
3	*Adathoda vasica*	0.99

TABLE 1: THE PHYTOCHEMICAL ANALYSIS OF ACETONE EXTRACT OF *SIDA CARDIFOLIA, PHYLLANTHUS AMARUS* AND *ADATHODA VASICA*

Tests	*Sida cardifolia*	*Phyllanthus amarus*	*Adathoda vasica*
Alkaloids			
Mayers test	+	+	+
Wagners test	+	+	+
Dragendroffs test	+	+	+
Carbohydrates			
Molisch tests	+	+	+
Fehling test	+	+	+
Benedict test	+	+	+
Flavonoids			
Shinoda test	+	+	+
Lead acetate test	+	+	+
Alkaline reagent test	+	+	+
Saponins			-
Foam test	-	-	-
Froath test	-	-	
Steroids			
Salkowski's test	+	+	+
Tannins			
Gelatin test	+	+	+
Anthocyanin	+	+	+
Phenols			
Ferric chloride test	+	+	+

Determination of total Phenolics by Folin-Ciocalteau assay:

The concentration of total Phenolics in the acetone leaf extracts of *Sida cardifolia, Phyllanthus amarus* and *Adathoda vasica* was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium [8]. The phenolic contents of the extracts were determined from calibration curve and were expressed in mg gallic acid equivalent/g of extract powder.

Determination of total sugars by Phenol-Sulphuric acid method:

Carbohydrate content of all the extracts at 100µg concentration was determined by the phenol-sulphuric acid method.

Estimation of total flavonoids:

Aluminum chloride colorimetric method was used for flavonoids determination[9]. The content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder

Determination of total Proanthocyanidins:

Total proanthocyanidin was determined for all the three acetone extracts based on the procedure of Sun et al [10]. Total proanthocyanidin content was expressed as gallic acid equivalent (mg/g) from the standard curve.

Determination of Tannins:

The tannin concentration was determined for each extract variety following a modified version of the vanillin-HCl method [11].

Determination of reducing power:

The reducing power of all the three acetone extracts was evaluated according to the method of Oyaizu [12].

Antioxidant activity by DPPH method:

2, 2-Diphenyl -1- picrylhydrazyl radical ($DPPH^{\cdot}$) was used as a stable radical for assessing antioxidant activity as described by Blios [13]. Reduction of DPPH by an antioxidant or by a radical species results in a loss of absorption at 517nm. Thus the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. Determination of antioxidant activity by the DPPH method was done for all the three acetone leaf extracts at 100µg concentration. Changes in the absorbance of the samples were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

Percentage of radical scavenging activity = Control OD-sample OD/Control OD

Antioxidant activity by TBA method:

Antioxidant activity of all three acetone extracts was performed using thio barbituric acid (TBA) according to the protocol of Halliwell and Gutteridge [14]. Lipid peroxidation induced by ferric chloride resulted in the production of malondialdehyde (MDA), lipid peroxide. Thio-barbituric acid (TBA) reacts with malondialdehyde (MDA) to form a di-adduct, a pink chromogen, which can be detected spectrophotometrically at 532nm.

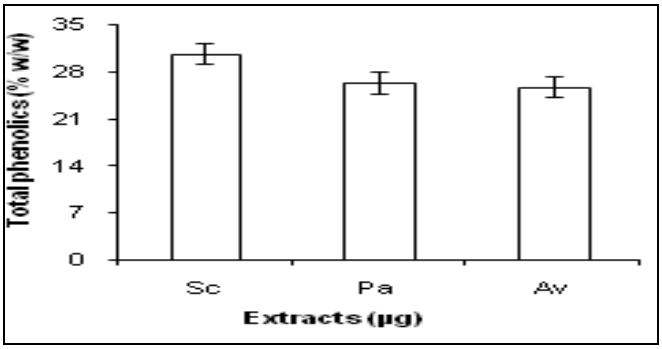

FIGURE1: ESTIMATION OF TOTAL PHENOLICS (% w/w) IN ACETONE EXTRACTS OF *SIDA CARDIFOLIA (SC), PHYLLANTHUS AMARUS (PA) AND ADATHODA VASICA (AV).* DATA REPRESENTS MEAN ±SD (n=3).

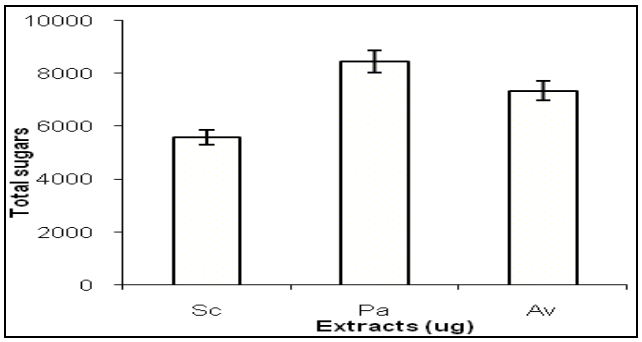

FIGURE 2: ESTIMATION OF TOTAL SUGARS IN ACETONE EXTRACTS OF *SIDA CARDIFOLIA (SC), PHYLLANTHUS AMARUS (PA) AND ADATHODA VASICA (AV).* DATA REPRESENTS MEAN ±SD (n=3).

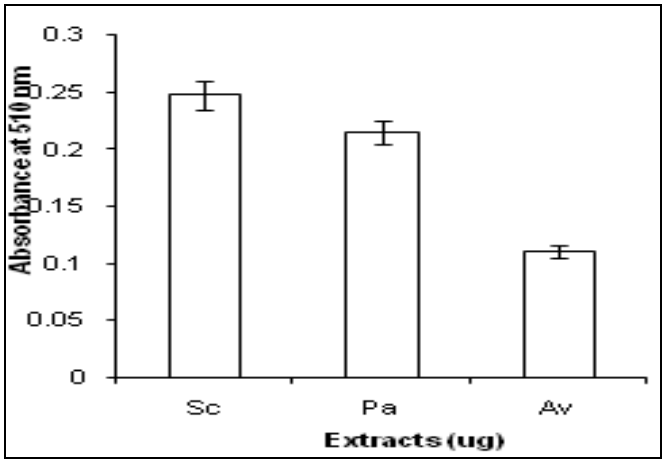

FIGURE 3: ESTIMATION OF TOTAL FLAVONOIDS IN ACETONE EXTRACTS OF *SIDA CARDIFOLIA (SC), PHYLLANTHUS AMARUS (PA) AND ADATHODA VASICA (AV)*. DATA REPRESENTS MEAN ±SD (n=3).

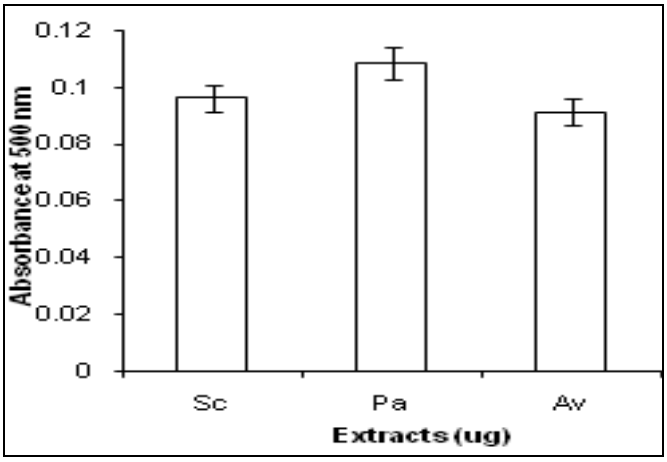

FIGURE 4: ESTIMATION OF TOTAL PROANTHOCYANIDINS IN ACETONE EXTRACTS OF *SIDA CARDIFOLIA (SC), PHYLLANTHUS AMARUS (PA) AND ADATHODA VASICA (AV)*. DATA REPRESENTS MEAN ±SD (n=3).

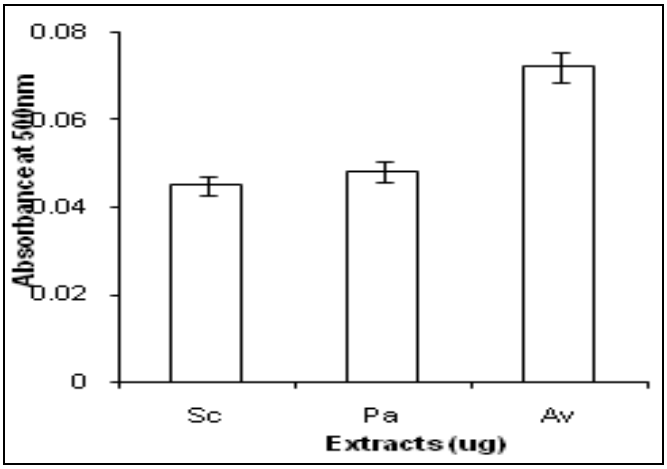

FIGURE 5: ESTIMATION OF TOTAL TANNINS IN ACETONE EXTRACTS OF *SIDA CARDIFOLIA (SC), PHYLLANTHUS AMARUS (PA) AND ADATHODA VASICA (AV)*. DATA REPRESENTS MEAN ±SD (n=3).

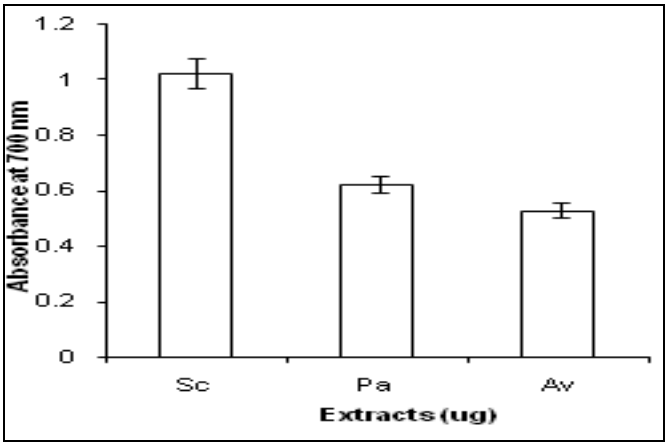

FIGURE 6: REDUCING POWER ACTIVITY IN ACETONE EXTRACTS OF *SIDA CARDIFOLIA (SC), PHYLLANTHUS AMARUS (PA) AND ADATHODA VASICA (AV)*. DATA REPRESENTS MEAN ±SD (n=3).

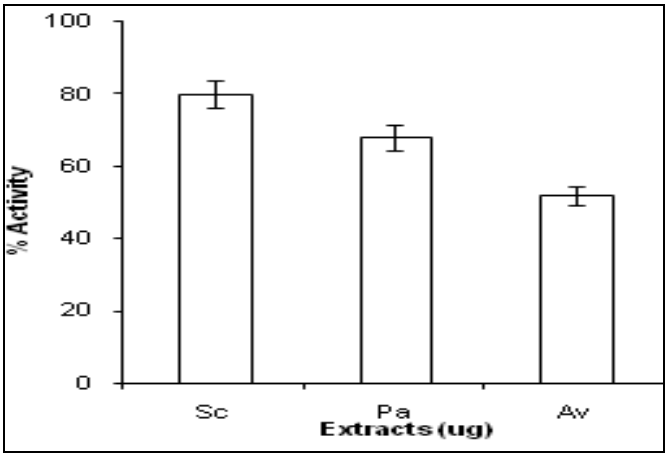

FIGURE 7: ANTIOXIDANT ACTIVITY BY DPPH METHOD IN ACETONE EXTRACTS OF *SIDA CARDIFOLIA (SC), PHYLLANTHUS AMARUS (PA) AND ADATHODA VASICA (AV)*. DATA REPRESENTS MEAN ±SD (n=3).

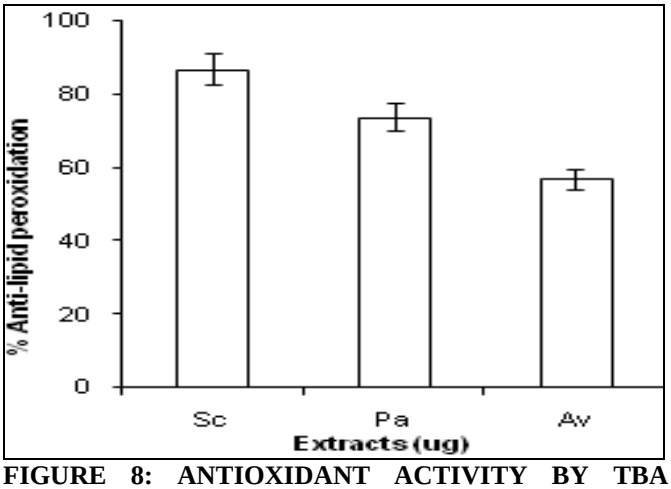

FIGURE 8: ANTIOXIDANT ACTIVITY BY TBA METHOD IN ACETONE EXTRACTS OF *SIDA CARDIFOLIA (SC), PHYLLANTHUS AMARUS (PA) AND ADATHODA VASICA (AV)*. DATA REPRESENTS MEAN ±SD (n=3).

RESULTS AND DISCUSSION:

The plant products over synthetic compound in the treatment of diseases are needed because of no deleterious effects on man. India is a home to a variety of traditional medicine system that relay to a very large extent on native plant species for their raw drug materials. Therefore there is a need to look backwards towards folk medicines which can serve as novel therapeutic agent. Many secondary metabolites grouped under polyphenols, terpenes and alkaloids exhibit antioxidant activity. The free radical intermediates and ROS escape from the site of reaction and act on various biological molecules such as lipids, nucleic acids, proteins and carbohydrates, thus causing deleterious changes in their structure and function and finally leading to cell death[13].

The percentage of yield (w/w) was calculated for *Sida cardifolia, Phyllanthus amarus and Adathoda vasica* acetone extracts as depicted in **Table 1**. The yield obtained from these extracts ranged from 0.99 to 4.09 %. The highest yield was obtained with acetone extract of *Phyllanthus amarus.*

It is well known that plant produce these metabolites to protect itself but recent research demonstrates that different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well being. It is well known that plant produce these metabolites to protect itself but recent research demonstrates that The qualitative analysis of the extracts from the leaf sample of *Sida cardifolia, Phyllanthus amarus and Adathoda vasica* showed the presence of phytochemical constituents such as alkaloids, carbohydrates, flavonoids, steroids, tannins, anthocyanin's and Phenolics. At the same time, the phytochemical constituent such as saponins was found to be absent **Table 2**. Phenolics and flavonoids were found to be more in *Sida cardifolia.* Total sugars and Proanthocyanidins in *Phyllanthus amarus* and tannins in *Adathoda vasica* respectively.

Phenolic compounds are naturally occurring secondary metabolites that are of great pharmacological interest. Total Phenolics was estimated for all the three acetone leaf extracts of *Sida cardifolia, Phyllanthus amarus and Adathoda vasica* at 100 µg concentration as shown in **Figure 1**. Out of the 3 acetone leaf extracts, acetone extract of *Sida cardifolia* with 1.53 mg gallic acid equivalent/g of extract powder showed maximum amount of Phenolics followed by the *Phyllanthus amarus and Adathoda vasica* with 1.325 and 1.29 mg gallic acid equivalent/g of extract powder. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties. These results gives a reason for the activity of these plants as antioxidant and how these plants extracts enable to scavenge the free radicals.

The acetone leaf extracts of *Sida cardifolia, Phyllanthus amarus and Adathoda vasica w*as used for of total sugars estimation at 100 µg concentration as shown in **Figure 2**. The acetone extract *Phyllanthus amarus* with 8.45 µg glucose equivalent/g of extract powder showed maximum amount of sugars followed by the and *Adathoda vasica* and *Sida cardifolia* with 7.34 and 5.60 µg glucose equivalent/g of extract powder.

The total flavonoid content of all the three was 7.09, 6.14 and 3.14 mg gallic acid equivalent/g of extract powder as shown in **Figure 3** with *S.* cardifolia acetone leaf extract maximum followed by *P. amarus* and *A. vasica.* These results gives a reason for the activity of these plants as antioxidant and how these plants extracts enable to scavenge the free radicals. Flavonoids exhibit various biological effects from their antioxidant properties.

Tannins are another major group of polyphenols in our diets and usually subdivided into two groups: (1) hydrolysable tannins and (2) condensed tannins. Researchers and food manufacturers have become more interested in polyphenols due to their potent antioxidant properties, their abundance in the diet, and their credible effects in the prevention of various oxidative stress associated diseases. The proanthocyanidin and tannin content was determined for all the three acetone leaf extracts and the results were as shown in Figure.4 and **Figure 5** respectively. *P.amarus* with 10.84 mg gallic acid equivalent/g of extract powder was found to be high in proanthocyanidins followed by *Sida cardifolia* and *A.vasica.* Tannin content was found to be high in *A. vasica* with 11.07 mg gallic

acid equivalent/g of extract powder and *P.amarus* with 7.4 and *S.cardifolia* with 6.92 mg gallic acid equivalent/g of extract powder

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. **Figure 6** shows the reducing power activity of *S.cardifolia*, *P.amarus* and *A.vasica* extracts at 100 µg concentration using the potassium ferricyanide reduction method. At 100 µg concentration, the extracts showed absorbances of 1.024, 0.623 and 0.529 respectively at 700 nm. Thus, the highest reducing activity was observed in *Sida cardifolia* acetone leaf extract. The reducing power activity is due to the presence of reductones (Phenolics).

Free radical scavenging potentials of all the three acetone leaf extracts at 100 µg concentrations were tested by the DPPH method and the results are shown in **Figure 7** *S.cardifolia*, *P.amarus* and *A.vasica* exhibited 80 %, 68 % and 52 % free radical scavenging activity respectively according to the DPPH method. DPPH is a stable radical that has been used to evaluate the antioxidant activity of rhizome extract. Antioxidant reacts with DPPH, which is a stable free radical, and converts it to α, α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The activity of extracts is attributed to their hydrogen donating ability. Increasing the number of hydroxyl or catechol groups increases radical scavenging activity. In presence of other H-donating groups (sulfhydryl, amide) in molecule also accelerates this activity.

The results of the effect of various leaf extracts at 100 µg concentration to prevent lipid peroxidation are shown in **Figure 8.** At 100 µg concentration, the *S.cardifolia, P.amarus and A.vasica* showed 87 %, 74 % and 57 % of inhibition of lipid peroxide generation by this method. Determination of the lipid peroxide content was carried out indirectly by means of derivatizing MDA with TBA at high temperature and acidic conditions. In biological systems, MDA is a very reactive species and takes part in cross-linking of DNA with proteins and also damages the liver cells. The production of lipid peroxides by ferrous/ascorbate systems in liver homogenates were inhibited greatly by the acetone leaf extract of *S.cardifolia, P.amarus and A.vasica*. If radicals initiate lipid peroxidation then the ability of antioxidants to scavenge radicals within the hydrophobic core of the membrane may explain their desirable therapeutic effect.

CONCLUSION: Thus acetone leaf extract of *Sida cardifolia* had maximum amount of polyphenols and flavonoids which is directly related to their greater antioxidant activity also. Thus active molecules present in the acetone leaf extract of Sida cardifolia has antioxidant property which may be useful in targeting the release of free radical intermediates along with the generation of ROS from various metabolic activities. They can be used for clinical development.

ACKNOWLEDGEMENT: The authors are very thankful to University Grants Commission, New Delhi, for financial assistance in the form of minor research project.

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How to cite this article:
Nanda BL and Radhakrishnan TT: A Comparitive Study of Phytochemicals and Antioxidant activity among Traditional Medicinal Plants. Int J Pharm Sci Res 2015; 6(4): 1609-15.doi: 10.13040/IJPSR.0975-8232.6(4).1609-15.