INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 November, 2013; received in revised form, 26 February, 2014; accepted, 28 March, 2014; published 01 May, 2014

EVALUATION OF ANTIOXIDANT POTENTIALS OF SELECTED INDIAN MEDICINAL PLANTS

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Keywords:
Antioxidant assays; Medicinal plants; Phytochemical analysis; Radical scavenging activity.

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ABSTRACT: Oxidative stress is found to be one of the major causes of health hazards in the present global scenario. The traditional herbs and medicinal plants have been investigated for the presence of natural antioxidants, which have incredible effects in the prevention of various oxidative stress associated diseases. The objective of present investigation is to assess the antioxidant capacity and phytochemical analysis of selected endemic medicinal plants viz., *Abutilon indicum, Albezia odoratissima, Aglaia elaegnoides, Calicarpa tomentosa, Colebrookia oppostifolia* and *Pterocarpus marsupium.* The antioxidant potentiality of these medicinal plants was assayed by 1,1-Diphenyl-2-picryl hydrazyl (DPPH), hydroxyl, superoxide radical scavenging and lipid peroxidation assays, total antioxidant potential by Ferric reducing antioxidant power (FRAP) assay. Qualitative phytochemical analysis revealed that ethanolic bark extract of *A. odoratissima* showed positive results for the presence of alkaloids, phenols, flavonoids, tannins, anthraquinones and saponins. The plants with high phenol contents showed high antioxidant activity. The results showed that ethanolic bark extract of *A. odoratissima* exhibited highest antioxidant activity by possessing considerably high phenolic content.

INTRODUCTION: Over the centuries, plants have been one of the important sources of many traditional medicines throughout the world. India is considered as a vast repository of valuable medicinal and aromatic plant species that are used in traditional system of medicine. In India, approximately 3000 plant species have been reported to have medical properties. Especially, antioxidants derived from medicinal plants have been used in the prevention and treatment of oxidative stress related diseases [1].

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.5(5).1819-29

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

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(5).1819-29

In the recent years, oxidative stress is found to be one of the major causes of health hazards. It has been implicated in the etiology of numerous diseases and disorders such as Alzheimer's disease, neural disorders, cardiovascular diseases, mild cognitive impairment, Parkinson's disease; alcohol induced liver diseases, atherosclerosis, ulcerative colitis, several cancers and ageing [2].

Under normal physiological conditions, the production of reactive oxygen species (ROS) is balanced by the endogenous antioxidative defense system. However, the deleterious oxidative stress can be generated as a result of imbalance between the production and elimination of ROS. These ROS can adversely cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes, nucleotides in DNA and critical sulfhydryl bonds in proteins.

Antioxidants are believed to play a pivotal role in the protection of cells from the damage caused by ROS. Experimental and epidemiological studies indicate that, there is an inverse correlation between the consumption of antioxidant rich diet and progression of diseases [3]. Thus, in recent years, researchers have been searching for pharmacologically potent and non-toxic antioxidants from natural resources, especially edible or medicinal plants [4].

Many indigenous medicinal plants have been used popularly as folk medicine in India and other Asian countries. The bioactive principles have been reported in some of these Indian medicinal plants [5]. Secondary metabolites such as polyphenols have drawn increasing attention due to their potent bioactivities and their credible effects in the prevention of various oxidative stress associated diseases [6].

Thus, the objectives of the present study were evaluation of antioxidant activity and phytochemical analysis of some selected Indian medicinal plants which are being used traditionally: *Calicarpa tomentosa, Albezia odoratissima, Pterocarpus marsupium, Colebrookia oppostifolia, Aglaia elaegnoides and Abutilon indicum.*

MATERIAL AND METHODS:

Chemicals: 1, 1-Diphenyl-2-picryl hydrazyl (DPPH), 2-Thiobarbituric acid (TBA), Nitroblue tetrazolium chloride (NBT), Trichloroacetic acid (TCA), 2, 4, 6-tri-(2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma Chemicals Co. USA. Gallic acid, Folin- Ciocalteau reagent, other chemicals and solvents were obtained from Merck Co, India. All chemicals and solvents used in the present study were of the analytical grade.

Plant collection and Preparation of plant extracts: The plant parts were collected from Chintapalli forest of Eastern Ghats, Andhra Pradesh, India in the month of April, 2009. All the plant parts were authenticated by the experts in the Department of Botany, Andhra University, Visakhapatnam, and Andhra Pradesh, India. Completely shade dried plant materials were powdered using mechanical blender.

Extracts were prepared by maceration of 20g of each plant material in 100ml of n-hexane, ethyl acetate, ethanol and water separately. Extracts were filtered using Whatman No.1 filter paper followed by evaporation of the filtrate under vacuum using rotary evaporator. Different concentrations of plant extracts were prepared using aforesaid solvents for further analysis.

DPPH radical scavenging activity: DPPH radical scavenging activity was measured by using the method of Scherer and Godoy [7] with slight modification. To 5ml of ethanolic DPPH (0.004%) solution, 50µl of plant extract (various concentrations) was added and mixed vigorously. After 30 min of incubation at 37^{o}C in the dark, the absorbance was measured at 517 nm by using UV-Visible spectrophotometer. For control, plant extract was replaced by respective solvent. The decrease in the absorbance of DPPH solution by the plant extract indicates its radical scavenging activity.

The radical scavenging activity was calculated using the following formula: Percent (%) of DPPH radical scavenging activity = $[(A_0 - A_1)/ A_0] \times 100$. Where A_0 is the absorbance of DPPH solution without plant extract (control) and A_1 is the absorbance of DPPH solution in presence of plant extract. The efficacy of the plant extracts to scavenge DPPH radicals was expressed as IC_{50} i.e. concentration at which 50% of DPPH radical was scavenged. The IC_{50} value was obtained from linear regression analysis and antiradical power ($1/ IC_{50} \times 100$) was expressed [8].

Superoxide radical scavenging activity: The superoxide radicals were generated as described by Sabu and Ramadasan [9]. Briefly, the superoxide anions were generated by the addition of 0.4ml of 0.1mM Hydroxyl amine in a reaction mixture containing 1.0ml of 125mM Sodium Carbonate, 0.4ml 25µM NBT and 0.2 ml of 0.1mM EDTA. The superoxide radical scavenging activity was initiated by adding 0.5ml of plant extract at various concentrations. For control, the plant extract was replaced by ethanol. After 5 min of incubation at room temperature, the absorbance was measured at 560 nm. The decrease in absorbance by plant extract indicates it's of superoxide radical scavenging ability.

The percent of inhibition of superoxide radicals was calculated by the following equation: Percent (%) of superoxide radical scavenging activity = $[(A_0 - A_1)] / A_0] \times 100$. Where A_0 was the absorbance of control and A_1 was the absorbance in the presence of plant extract. The superoxide radical scavenging efficiency of the plant extracts was expressed as $IC_{50.}$

Hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity was carried out by the method of Vardar *et al* [10]. This method involves *in vitro* generation of hydroxyl radicals by Fenton reaction using Fe^{3+}/ Ascorbate / EDTA / H_2O_2 system. Briefly, 0.1ml of plant extract at various concentrations was added to the reaction mixture containing 0.1ml of 3mM Deoxy ribose, 0.5ml of 0.1mM $FeCl_3$, 0.5ml of 0.1mM EDTA, 0.5ml of 0.1mM Ascorbic acid, 0.5ml of 0.1mM H_2O_2 and 0.8ml of 20mM Phosphate buffer, pH 7.4 in a final volume of 3ml. After incubation at 37^{o}C for 1hr, 1ml of 1% TBA and 1ml of 2.8% TCA were added and kept the reaction mixture in water bath for 20 min at 100^{o}C.

The reaction mixture was cooled and absorbance was measured at 532 nm against control. For control, plant extract was replaced by ethanol. The percent of hydroxyl radical scavenging activity of extracts was calculated according to the following equation: Percent (%) of hydroxyl radical scavenging activity = $[(A_0 - A_1)] / A_0] \times 100$. Where A_0 was the absorbance of control and A_1 was the absorbance in the presence of plant extracts. The hydroxyl radical scavenging activity of plant extracts were expressed as $IC_{50.}$

Inhibition of lipid peroxidation: To study the inhibition of lipid peroxidation by the plant extracts, lipid peroxidation was induced by $FeSO_4$ – Ascorbate system in sheep liver homogenate by previously described method of Bishayee & Balasubramaniyam [11]. The degree of lipid peroxidation was assayed in terms of thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa *et al* [12]. The inhibition of lipid peroxidation was initiated by adding 0.4ml of plant extract at various concentrations to the reaction mixture containing 0.1ml of 25% (w/v) sheep liver homogenate in 40mM Tris –HCl buffer (pH 7.0), 30mM KCl, 0.16mM $FeSO_4$ and 0.06mM Ascorbic acid. The reaction mixture was then incubated at 37^o C for 1 hr. Then, 0.4ml of reaction mixture was treated with 0.2ml of 8.1% SDS, 1.5ml of 1% TBA and 1.5ml of 20% acetic acid and the pH was adjusted to 3.5. The total volume was then made up to 4.0ml by adding distilled water and the reaction mixture was kept in a water bath at 95^o C for 1hr. After cooling to RT, 1ml of distilled water and 5ml of n-butanol and pyridine mixture (15:1 v/v) were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the upper organic layer was taken and its absorbance was measured at 532nm. For control, plant extract was replaced by ethanol. The percent of inhibition of lipid peroxidation was calculated according to the following equation: Percent (%) of inhibition of lipid peroxidation = $[(A_0 - A_1)] / A_0] \times 100$. Where A_0 was the absorbance of control and A_1 was the absorbance in the presence of plant extracts at different concentrations. The results were expressed as IC_{50}.

Total Antioxidant Power Assay: The total antioxidant power of the plant extracts was assayed by the modified FRAP method [13]. Briefly, the FRAP reagent was prepared by using 300mM acetate buffer (pH 3.6), 10mM TPTZ solution in 40mM HCl and 20mM $FeCl_3.6H_2O$ in 10:1:1 ratio respectively. The freshly prepared FRAP reagent was warmed in water bath 37^0 C at before use. 100µl of the plant extract at various concentrations was added to 3ml of FRAP reagent. The absorbance of colored product (Ferrous tripyridyl triazine complex) was recorded at 593nm against the reagent blank after 4 min. The standard curve (Y = 0.008 + 0.002X, R=0.999) was constructed using iron (II) sulphate solution in the range of 0-1000µM. The results were expressed as mM Fe (II) equivalents antioxidant capacity per gram of dry weight.

Preliminary phytochemical analysis: Phyto-chemical analysis of the ethanolic extracts of selected medicinal plants was carried out to detect the presence of different phytoconstituents by using standard qualitative tests [14].

Determination of total phenolics: The total phenolics were determined by Folin-Ciocalteu method [15]. To 0.5ml of each plant extract (1000µg/ml), 5ml of diluted Folin-Ciocalteu

reagent (3:1) and 4ml aqueous 1M Na_2CO_3 were added. The mixture was allowed to stand for 15 min and absorbance was measured at 760 nm. The total phenolic content was calculated using Gallic acid standard curve (Y=0.03+0.02X, R^2 =0.9998). The results were expressed as mg (GAE)/gm dry weight of plant material.

RESULTS AND DISCUSSION: In spite of spectacular advance in pharmaceuticals and modern medical research, 80% of world population still relies on plant based medicines [1]. As plants produce significant amounts of bioactive compounds with biological activities, they serve as leads for the sustainable exploration of new therapeutic drugs [16]. Considering an upsurge of interest in the natural antioxidants, phytochemical and the antioxidant analysis of some selected medicinal plants were carried out. Different free radical generating systems were employed in this study to assess the antioxidant capacity of medicinal plants.

The antioxidant activity and the extraction of phytochemicals were invariably affected by the polarity of the solvents [17]. Therefore, to identify suitable solvent for optimum extraction of potential bioactive compounds, solvents with different polarities (n-Hexane, Ethyl acetate, Ethanol and Water) were used to prepare medicinal plant extracts.

Radical scavenging ability of the plants: DPPH, a stable and artificial free radical is extensively used to screen the radical scavenging ability of plant extracts. It is sensitive and very convenient assay [18]. As paramagnetic and nitrogen centered DPPH radical can accept electron or hydrogen to become stable diamagnetic molecule, the ability of the medicinal plant extracts to scavenge reactive oxygen species by donating hydrogen or electron was measured through the DPPH radical scavenging assay. The antioxidant activity of plant extracts depends on their hydrogen donating ability which is an indication of the capacity to scavenge free radicals. IC_{50} value is the concentration required to inhibit 50% DPPH radicals. The lower IC_{50} values indicate the greater antioxidant activity.

Antiradical power ($1/IC_{50}\times100$) is a good measure of the antioxidant efficiency of plant extracts. The results showed that the highest ARP was evident in ethanolic extracts, compared to other solvent extracts of medicinal plants (**Fig. 1**). The order of antiradical power of different solvent extracts of plants was found to be as follows, Ethanol > Water > Ethyl acetate > n-Hexane. The high radical scavenging activity with ethanolic extracts may be due to its rapid electron and proton transfer capacity and quick proton releasing capacity of ethanol compared to non-ionizing solvents such as water, ethyl acetate and n-hexane [19].

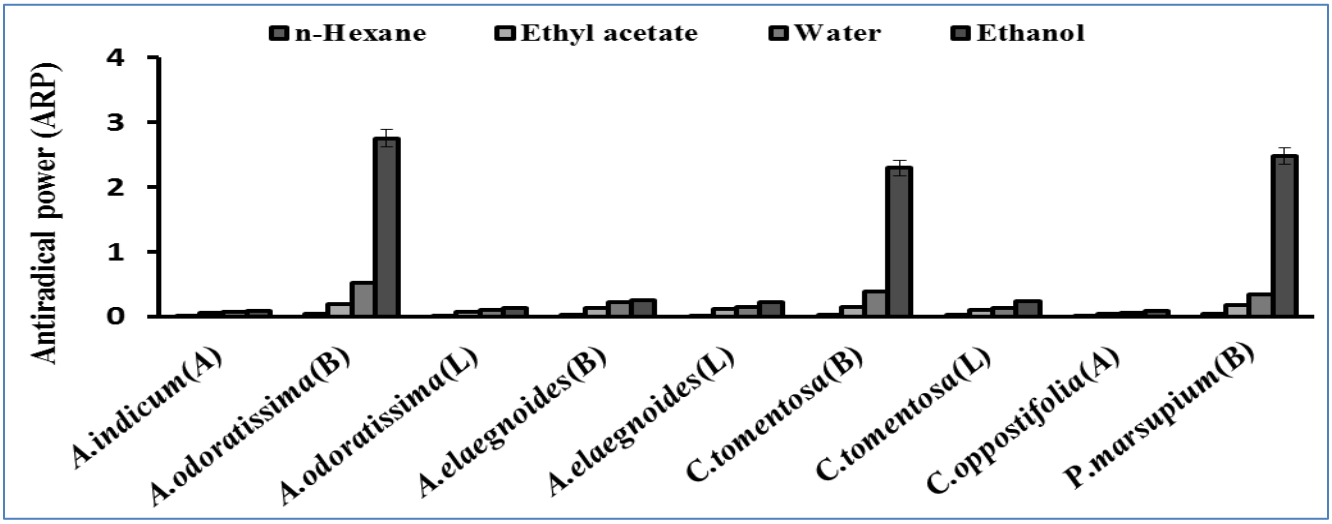

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF SELECTED MEDICINAL PLANTS WAS DETERMINED AS DESCRIBED IN "MATERIALS AND METHODS". The results were expressed in Antiradical power (ARP). Each experiment was repeated for three times. Values were expressed as mean ± S.D (n=3). Two way analysis of variance (ANOVA) was carried out to test significant differences among the plant species (*P<0.05) and different solvent extracts (*P>0.05). A-aerial, B-bark and L-leaf.

Therefore ethanol, an organic and non toxic solvent, is considered as good solvent for maximum extraction of antioxidant compounds. The ARP values of ethanolic extracts of bark of *A. odoratissima*, *P. marsupium*, *C. tomentosa*, *A. elaegnoides*, leaves of *C. tomentosa, A.elaegnoides*, *A. odoratissima* and aerial parts of *C. oppostifolia* and *A. indicum* were found to be 2.75, 2.49, 2.29, 0.25, 0.23, 0.22, 0.13, 0.09 and 0.09 respectively. Among the ethanolic extracts, *A. odoratissima* bark exhibited highest antiradical capacity with lowest IC_{50} value (36.32µg/ml). The results of present study indicate that DPPH radical scavenging activity of ethanolic bark extract of *A. odoratissima* was significantly higher than bark extract of *A. adianthifolia* [20], leaf extract of *A. procera* [21] and bark extract of *A. lebbeck* [22] and similar with bark extract of *A. zygia* [23].

Superoxide radical **($O_2^{\bullet-}$)**, the primary ROS, is moderatively reactive, short lived with a half life of approximately 1µs. Therefore, it has low capacity to penetrate lipid membrane layer. Furthermore, it is easily dismutated to H_2O_2, which can lead to the production of hydroxyl radicals, the most reactive and deleterious oxidant in the ROS family. $O_2^{\bullet-}$can also react with nitric oxide (NO) to give rise to peroxy nitrate ($OONO^-$) which is potent oxidant that causes nitrosative stress in the organ systems [24]. Therefore, it is of great importance to scavenge superoxide anion radicals. In the present study, the super oxide radical scavenging (SRSA) ability of ethanolic extracts of selected medicinal plants was evaluated using Hydroxyl amine/EDTA system by measuring the reduction of yellow colored NBT to blue formazan.

The results showed that all the extracts exhibited $O_2^{\bullet-}$ scavenging activity in a dose dependent manner (**Fig. 2**). The scavenging activity of ethanolic bark extract of *A. odoratissima* against superoxide radicals was 55.49% at 50 µg/ml and 90.66% at 1000µg/ml. The ethanolic bark extracts of *P. marsupium*, *C. tomentosa*, *A. elaegnoides*, leaf extracts of *A. odoratissima* and *A. elaegnoides* exhibited moderate superoxide anion radical activity with 37.09, 22.44, 17.4, 13.45 and 9.66 % respectively, at 50 µg/ml and 87.59, 77.27, 66.02, 58.67 and 54.88 % respectively, at 1000 µg/ml.

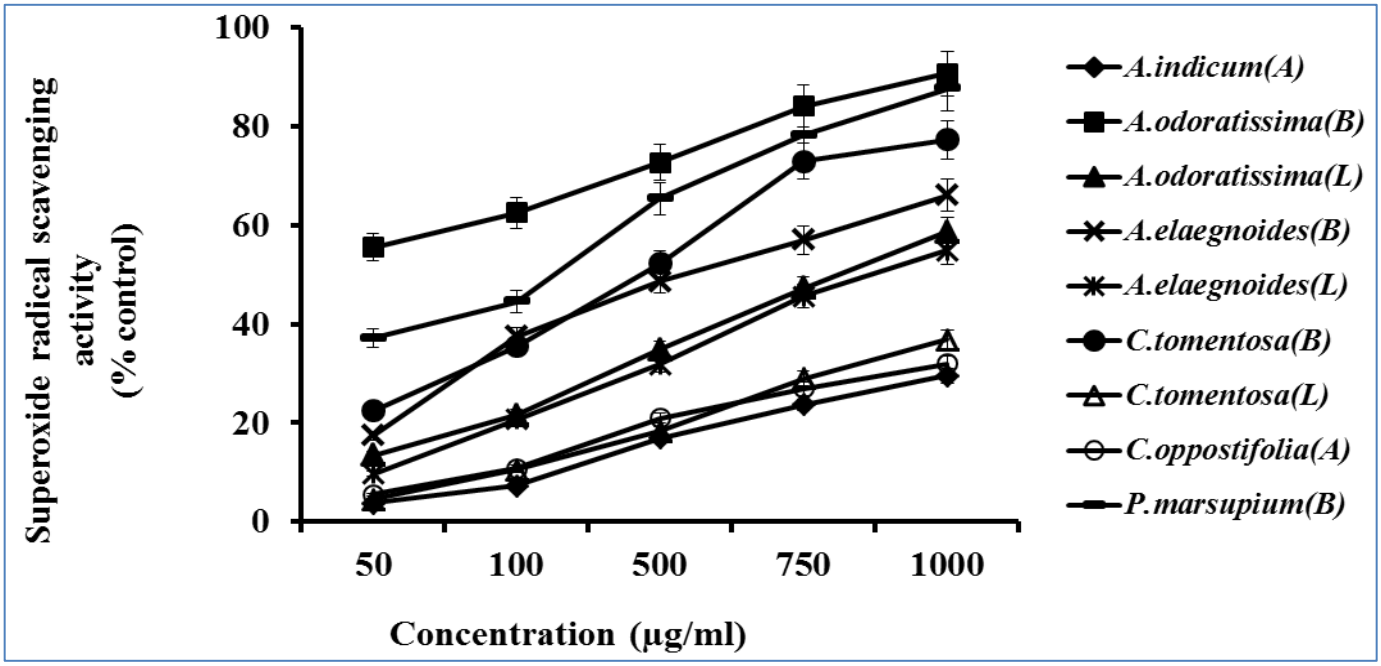

FIG. 2: Ethanolic extracts of selected medicinal plants in the concentration range of 50-1000 µg/ml were used to evaluate superoxide radical scavenging activity as described in "Materials and Methods". The results were expressed in percent control. Each experiment was repeated for three times. Values were expressed as mean ± S.D (n=3). Two way analysis of variance (ANOVA) was carried out to test significant differences among the plant species and different concentrations of plant extracts and was significant at *P > 0.05. A-aerial, B-bark and L-leaf.

The superoxide radical scavenging activities of ethanolic extracts of leaves of *C. tomentosa* (4.55 to 36.87%) and aerial parts of *C. oppostifolia* (5.5 to 32%) and *A. indicum* (3.78 to 29.56 %) at the concentration range 50 to 1000 µg/ml were significantly less as compared with ethanolic bark extract of *A. odoratissima*. The IC_{50} values of ethanolic extracts of bark of *A. odoratissima*, *P. marsupium*, *C. tomentosa*, *A. elaegnoides*, leaves of *A. odoratissima*, *A. elaegnoides*, *C. tomentosa* and aerial parts of *C. oppostifolia* and *A. indicum* were found to be 43, 239, 443, 589, 813, 879, 1428, 1764 µg/ml, respectively. These results indicate that ethanolic bark extract of *A. odoratissima* was an effective scavenger of superoxide anion radicals with lowest IC_{50} value. Therefore, it might be beneficial for preventing injury induced by superoxide radicals in pathological conditions.

Hydroxyl radicals ($OH^{\bullet}$) are highly potent oxidants with estimated half life of 10^{-9} sec. These are highly damaging species in free radical pathology and react with almost all adjacent biomolecules such as proteins, nucleic acids, polyunsaturated fatty acids (PUFA) at a very high rate. This leads to development of cancers, premature ageing and other diseases. Hydroxyl radicals which are formed from the radiolysis of water are responsible for 60-70% of cellular DNA damage produced by ionizing radiations [25].

Therefore, removal of hydroxyl radical is one of the most important defense mechanism of living systems against various diseases.

In the present study, hydroxyl radicals generated from Fenton reaction was used to evaluate the scavenging ability of ethanolic extracts of selected medicinal plants. The data presented in the **fig. 3** describes the hydroxyl radical scavenging abilities of ethanolic extracts of selected Indian medicinal plants. The hydroxyl radical scavenging activities of ethanolic bark extracts of *A. odoratissima* (50.59 – 88.24 %), *P. marsupium* (33.24 – 84.65 %) and *C. tomentosa* (30.42 – 82.46 %) were increased with increased concentration from 50 to 500µg/ml.

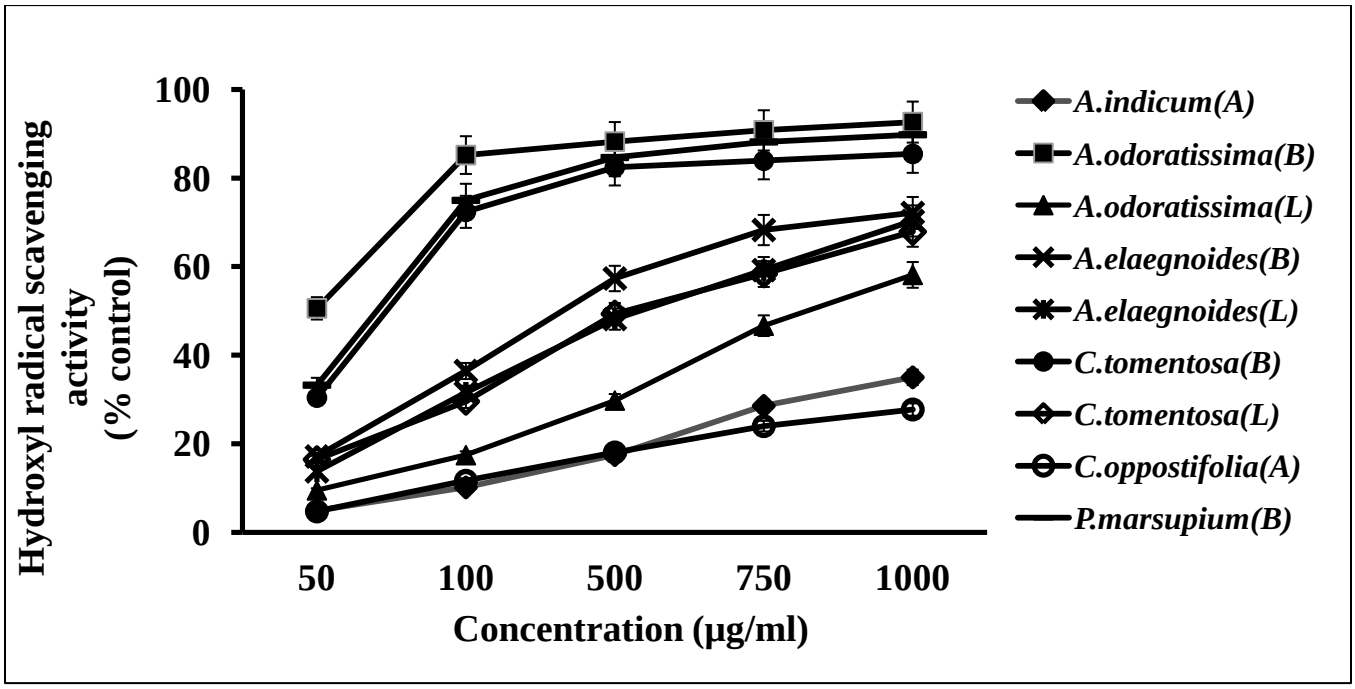

FIG. 3: ETHANOLIC EXTRACTS OF SELECTED MEDICINAL IN THE CONCENTRATION RANGE OF 50-1000 µg/ml WERE USED TO EVALUATE HYDROXYL RADICAL SCAVENGING ACTIVITY WAS DETERMINED AS DESCRIBED IN "MATERIALS AND METHODS". The results were expressed in percent control. Each experiment was repeated for three times. Values were expressed as mean ± S.D (n=3). Two way analysis of variance (ANOVA) was carried out to test significant differences among the plant species and different concentrations of plant extracts and was significant at*P > 0.05. A-aerial, B-bark and L-leaf

Further the activity was slightly raised to 92.65, 89.79 and 85.46 % at 1000 µg/ml. Ethanolic extracts of both bark and leaf of *A.elaegnoides*, leaves of *C. tomentosa* and *A. odoratissima* exhibited moderate hydroxyl radical scavenging activities with 17.14, 13.78, 16.46 and 9.48 % respectively, at 50 µg/ml and further increased to 72.13, 70.33, 67.89 and 58.14 % respectively, at 1000 µg/ml. Ethanolic extracts of aerial parts of *A. indicum* (4.87 – 35 %) and *C. oppostifolia* (*4.72* - 27.74 %) showed significantly less hydroxyl radical scavenging activities in the concentration ranged from 50-1000 µg/ml. The IC_{50} values of ethanolic bark extracts of *A. odoratissima, P. marsupium, C. tomentosa, A.elaegnoides*, leaf extracts of *A.elaegnoides, C. tomentosa, A. odoratissima* and aerial parts extracts of *A. indicum* and *C. oppostifolia* were 36.5, 40.2, 47.3, 475, 593, 844, 1500, 1969 µg/ml, respectively. As *A.odoratissima* displayed significantly lowest IC_{50} values, it may be considered as potent hydroxyl radical scavenger and may provide better protection to cells against damage caused by hydroxyl radicals.

Lipid peroxidation is a free radical mediated process in which oxidative damage leads to destabilization and disintegration of the cell membrane. This causes liver injury, atherosclerosis, kidney damage, aging and increased susceptibility to cancer [26]. Polyunsaturated fatty acids (PUFA) found in biological membranes are particularly vulnerable to the lipid peroxidation process because of the multiple unsaturation points found along their backbone. A single $OH^{\bullet}$ can cause peroxidation of several PUFA in a cyclic chain reaction. The most widely used model to evaluate lipid peroxidation is the use of TBARS such as malondialdehyde (MDA), 4-Hydroxy nonenal (4-HNE) and various 2- alkenals. The formation of TBARS is an index of lipid breakdown [27]. Thus, their reduction would indicate inhibition of lipid peroxidation.

In the present study, $FeSO_4$-ascorbic acid system induced lipid peroxidation in sheep liver homogenate was used as model to evaluate the lipid peroxidation inhibitory activity of ethanolic medicinal plant extracts (**Fig. 4**).

The results indicate that ethanolic extracts of selected medicinal plants exhibited different levels of inhibition of TBARS formation. The ethanolic bark extracts of *A. odoratissima*, *P. marsupium*, *C. tomentosa* exhibited 52.45%, 37.45% and 28.03% of inhibition of lipid peroxidation at 50 µg/ml ,which further increased to 92.15, 88.59 and 86 15% respectively, at 1000 µg/ml. The ethanolic extracts of both bark and leaf of *A.elaegnoides*, leaf of *A. odoratissima* and *C. tomentosa* showed moderate inhibition of lipid peroxidation with 73.71%, 62.14%, 61.89% and 60.89% respectively, at 1000 µg/ml. Significantly, less inhibition of lipid peroxidation was observed with ethanolic extracts of aerial parts of *A. indicum* (32.87%) and *C. oppostifolia* (31.84%) at 1000 µg/ml, in comparison with other medicinal plant extracts. The IC_{50} values of ethanolic extracts of bark of *A. odoratissima*, *P. marsupium*, *C. tomentosa*, *A.elaegnoides*, leaves of *A. odoratissima, C. tomentosa, A.elaegnoides* and aerial parts of *A. indicum* and *C. oppostifolia* for inhibition of lipid peroxidation were 40.32, 201.3, 268, 518.4, 710.5, 717.4, 726.6, 1552, 1600 µg/ml, respectively.

These results indicate that ethanolic bark extract of *A. odoratissima* exhibited significant lipid peroxidation inhibiting activity with less IC_{50} value, suggesting that it may contain antioxidant compounds which are attributed to terminate lipid peroxidation.

Total antioxidant power assay: Ferric reducing antioxidant power (FRAP) assay is widely employed method for measuring the total antioxidant capacity of plant extracts. It is a simple, reproducible and inexpensive method. This is based on the reduction of ferric tripyridyl triazine (Fe^{3+} - TPTZ) to the intense blue color ferrous form with absorption maxima at 593nm. The intensity of color is proportional to the amount of antioxidants present in the extracts [28]. The results on total antioxidant activity of the ethanolic plant extracts reveals that the ethanolic extracts of *A. odoratissima* bark exhibited highest total antioxidant capacity (8.867mM Fe (II)/g) followed by *P. marsupium* bark (8.183mM Fe (II)/g). Moderate total antioxidant capacity was observed with ethanolic extracts of bark of *C. tomentosa*, both bark and leaf of *A. elaegnoides* with 5.75, 3.25

and 2.5mM Fe (II)/g of dry weight, respectively. The ferric reducing power of ethanolic extracts of leaves of *A. odoratissima*, *C. tomentosa*, aerial parts of *C. oppostifolia* and *A. indicum* were significantly less with 1.27, 0.95, 0.9 and 0.65mM Fe (II)/g of dry weight, respectively (**Fig. 5**). Among all plants, ethanolic bark extract of *A. odoratissima* found to possess the highest reducing power. It indicates that the antioxidant compounds present in this plant may be acting as electron donors thereby reducing free radical generation.

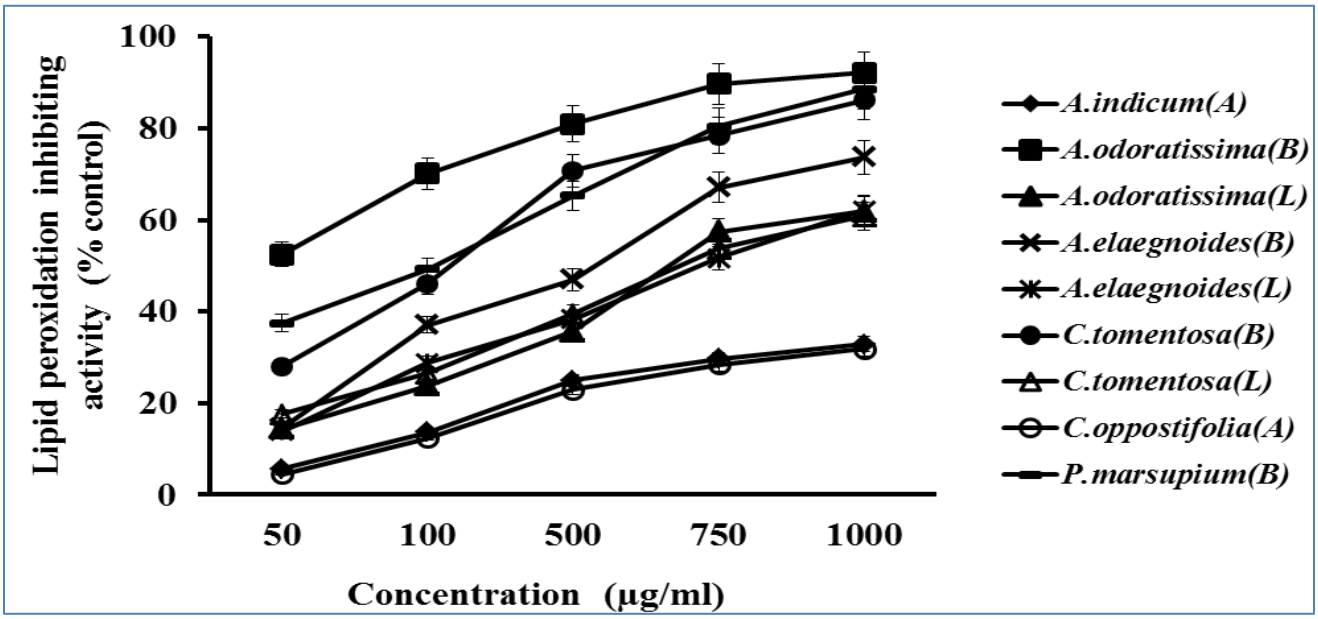

FIG. 4: ETHANOLIC EXTRACTS OF SELECTED MEDICINAL PLANTS IN THE CONCENTRATION RANGE OF 50-1000 µg/ml WERE USED TO EVALUATE INHIBITION OF LIPID PEROXIDATION WAS DETERMINED AS DESCRIBED IN "MATERIALS AND METHODS". The results were expressed in percent control. Each experiment was repeated for three times. Values were expressed as mean ± S.D (n=3). Two way analysis of variance (ANOVA) was carried out to test significant differences among the plant species and different concentrations of plant extracts and was significant at*P > 0.05. A-aerial, B-bark and L-leaf

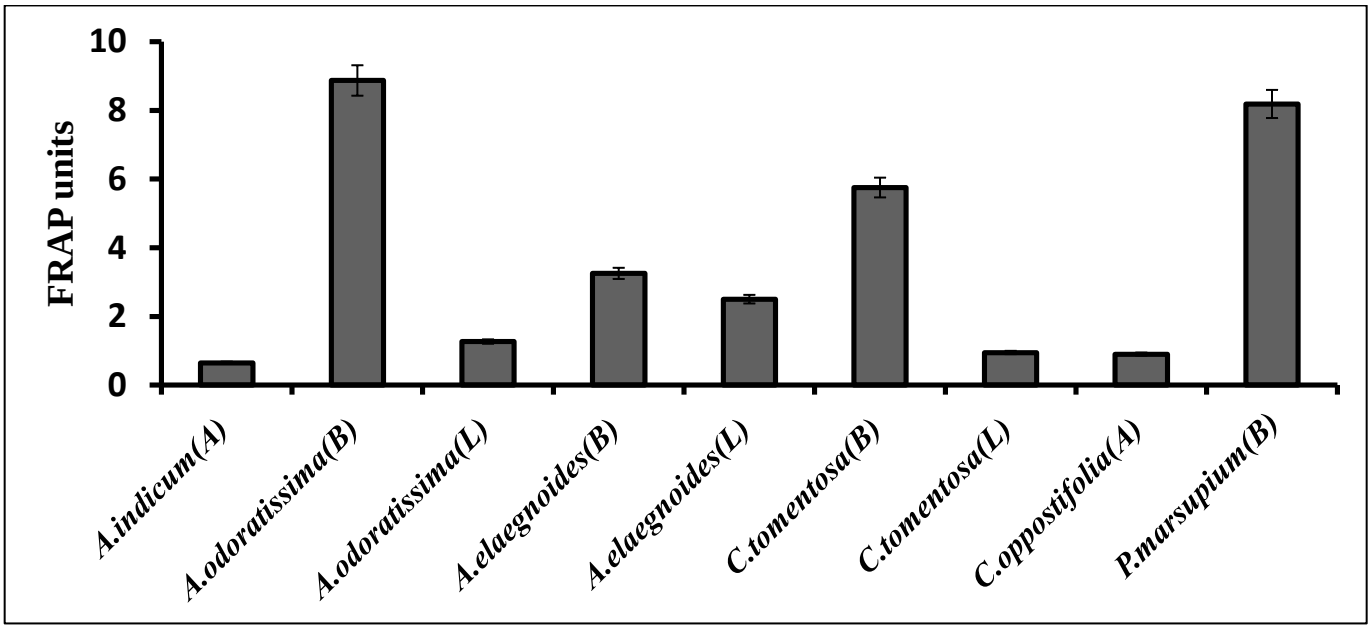

FIG. 5: ETHANOLIC EXTRACTS OF MEDICINAL PLANTS WERE USED TO DETERMINE TOTAL ANTIOXIDANT ACTIVITY BY FRAP METHOD AS DESCRIBED IN "MATERIALS AND METHODS". The results were expressed as mM Fe (II) equivalents antioxidant capacity per gram of dry weight (FRAP units). Each experiment was repeated for three times. Values were expressed as mean ± S.D (n=3). A-aerial, B-bark and L-leaf.

Phytochemical analysis of medicinal plant extracts: Phytochemical analysis of ethanolic extracts of medicinal plants using qualitative tests revealed that ethanolic bark extracts of *A. odoratissima*, *P. marsupium* and *C. tomentosa* showed positive results for the presence of alkaloids, phenols, flavonoids, tannins, anthraquinones and saponins **(Table 1).** Further, the intensity of color revealed that high levels of phenols, flavonoids and tannins, moderate levels of anthraquinones and low levels of alkaloids and saponins were present in ethanolic bark extract of *A. odoratissima.* Ethanolic bark extract of *P. marsupium* exhibited moderate levels of alkaloids, phenols, tannins, and anthraquinones, low levels of flavonoids and traces of saponins. However, the other ethanolic extracts of medicinal plants exhibited significantly low levels of all phytochemicals.

TABLE 1:

S. No.	Plant Name	Alkaloids	Poly phenolics	Flavonoids	Tannins	Anthraquinones	Saponins
1.	*A. indicum* (Aerial)	++	+	ND	+	ND	ND
2.	*A. odoratissima* (Bark)	++	++++	++++	+++	+++	++
3.	*A. odoratissima* (Leaf)	+	+	+	+	ND	ND
4.	*A. elaegnoides* (Bark)	ND	++	ND	++	ND	++
5.	*A. elaegnoides* (Leaf)	++	+	+	ND	ND	ND
6.	*C. tomentosa* (Bark)	++	++	++	+	+	+
7.	*C. tomentosa* (Leaf)	+	+	ND	+	ND	+
8.	*C. oppostifolia* (Aerial)	+++	+	+	+	ND	++
9.	*P. marsupium* (Bark)	+++	+++	++	+++	+++	+

Phytochemical analysis of ethanolic extracts of selected medicinal plants. Ethanolic extracts (1000 µg/ml) of selected medicinal plants were analyzed for the presence of different phytochemicals using standard qualitative test. Based on the color intensity, the results were expressed as arbitrary units high (++++), moderate (+++), low (++ or +), and not detected (ND).

Estimation of total phenolics In recent years, natural polyphenolic compounds, one of the ubiquitous groups of plant metabolites, become a major area of health and medical related research due to their potent antioxidant properties and their credible effects in the prevention of various oxidative stress associated diseases [6]. Preliminary phytochemical analysis revealed that polyphenols were present in all ethanolic extracts of medicinal plants used in the present study. Therefore, the total phenol content was evaluated using gallic acid standard curve **(Fig. 6).**

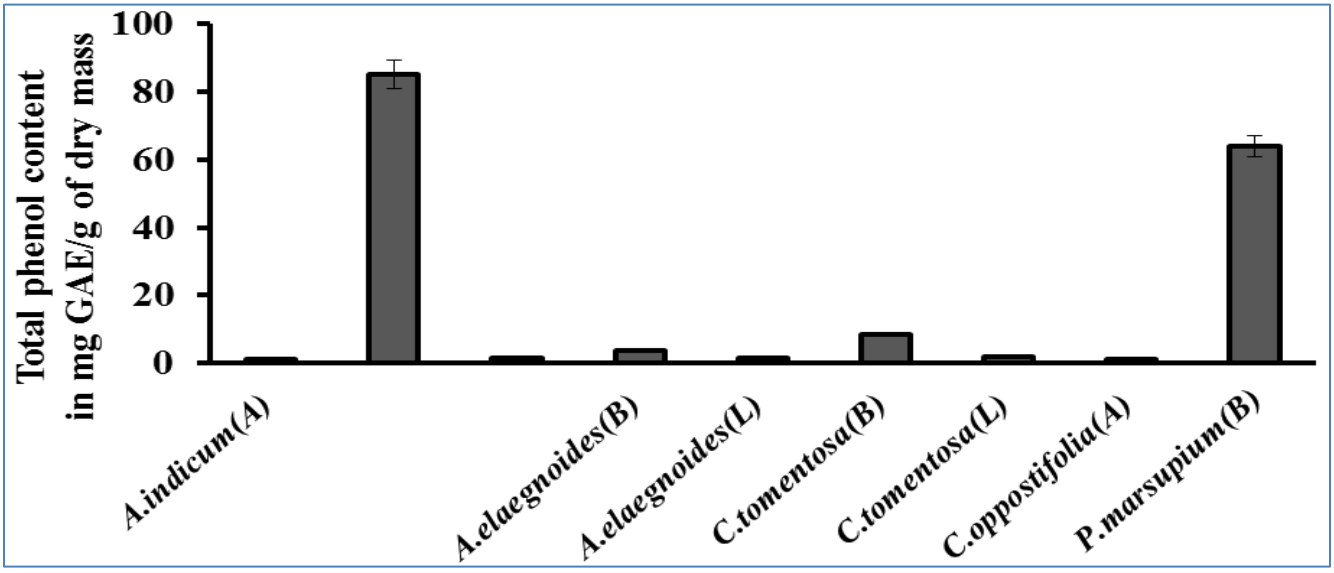

FIG. 6: ETHANOLIC EXTRACTS OF MEDICINAL PLANTS WERE USED TO DETERMINE TOTAL PHENOLIC CONTENT BY FOLIN-CIOCALTEU METHOD AS DESCRIBED IN "MATERIALS AND METHODS". The results were expressed as mg Gallic acid equivalents (GAE)/gram of the plant material (dry weight). Each experiment was repeated for three times. Values were expressed as mean ± S.D (n=3). A-aerial, B-bark and L-leaf.

The results showed that ethanolic bark extract of *A. odoratissima* was found to have the highest phenol content (85 mg GAE/g of dry plant material) followed by ethanolic bark extract of *P. marsupium* (64 mg GAE/g). Significantly, low phenol content was observed in ethanolic extracts of bark (9.44 mg GAE/g) and leaf (2 mg GAE/g) of *C. tomentosa*, bark (3.6 mg GAE/g) and leaf (1.5 mg GAE/g) of *A. elaegnoides*, leaf of *A. odoratissima* (1.34 mg GAE/g), aerial parts of *C. oppostifolia* (1.14 mg GAE/g) and *A. indicum* (1.02 mg GAE/g).

Earlier studies have been reported that there is a correlation between antioxidant activity and the amount of phenolic content of the plant extracts [29]. Muthu et al. [30] reported that ethanolic extracts of aerial parts of *A. procera* was a significant source of phenolic compounds. Krithika *et al* [31] reported that *A. saman* was rich source of phytochemicals and has high antioxidant property. It was reported that methanolic bark extract of *A. lebbeck* and leaf extract of *A. procera* showed high radical scavenging activity and contained high phenolic content 84.44 and 101.76 mg GAE/g [21, 22]. Accordingly, the present study revealed that the highest antioxidant activity of ethanolic bark extract of *A. odoratissima* might be attributed to the presence of phenolic compounds. The variations in antioxidant potential in different studies might be due to genotype, environmental differences within the species, the parts of the plant studied, time of sample collection and analytical methods used [32].

Statistical analysis: The results were analyzed statistically and expressed as mean ± standard deviation (SD) of three determinations. IC_{50} were calculated by linear regression analysis. Two way analysis of variance (ANOVA) was carried out to test significant differences among the plant species and at different concentrations.

CONCLUSION: Medicinal plants are good source of natural antioxidants. However, the nature and amount of antioxidant compounds extracted is varied from solvent to solvent. Different solvent extracts of medicinal plants viz., n-hexane, ethyl acetate, ethanol and water were evaluated by using DPPH radical.

The ethanolic extracts of all medicinal plants used in the present study exhibited significant DPPH radical scavenging activity. Among, the ethanolic bark extract of *A. odoratissima* showed potent radical scavenging and total antioxidant activities. It was also found to have highest phenolic content which might be responsible for its antioxidant activity. Therefore, further research on isolation of antioxidant compounds from this plant might be helpful in the development of drugs or functional foods for the prevention and treatment of diseases caused by oxidative stress.

ACKNOWLEDGEMENTS: The authors would like to thank management of M.V.R. Degree & P.G College for providing all necessary laboratory facilities to conduct this work. We also wish to thank eminent authorities of GITAM for generous support and encouragement.

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How to cite this article:
Kaja LK, Rao PVA, Kumar ADN, Babu BG and Malla RR: Evaluation of antioxidant potentials of selected indian medicinal plants. *Int J Pharm Sci Res* 2014; 5(5): 1819-29.doi: 10.13040/IJPSR.0975-8232.5 (5).1819-29.