Available online at http://www.ijcrls.com

International Journal of Current Research in Life Sciences
Vol. 07, No. 04, pp.1836-1841, April, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

ANTI-BACTERIAL AND FREE-RADICAL SCAVENGING ACTIVITY PLANT LEAF, STEM AND ROOT OF *ARGEMONE MEXICANA.* (L.)

***Pavithra, S., Dr. Sekar, T. and Sujatha, S.**

Department of Botany, Bharathiar University, Coimbatore, Tamilnadu – 641 046, India

Received 19th February, 2018; Accepted 13th March, 2018; Published 30th April, 2018

ABSTRACT

In the present study is investigated of antioxidant activities the pet-ether, chloroform, acetone and methanolic extracts from Argemone *mexicana. (L.)***.** Leaf, stem and root**.** Ayurvedic medicine of *A .mexicana* (L). Is used for healing wound, constipation, malaria, chronic, fever etc. The ability of the plant extract to act as hydrogen/electrons donor or scavenger of radicals were determined by in-vitro antioxidant assays using 2,2-diphenyl-2-picryl-hydrazyl free radical ($DPPH^{\cdot}$) scavenging, reducing power assay, superoxide radical (O_2^{*}) scavenging activity, phosphomolybdenum assay, and metal chelating activity were performed to know the antioxidant potency. Antibacterial studies were investigated the plant leaf methanolic extract of *A. mexicana*. Results are evaluated higher in leaves, stem and root of *A. mexicana* recorded total phenol (49.93±5.30), DPPH assay antioxidant activity leaf in methanolic extract of (10.25 µg/ml) and phosphomolybdenum assay in methanolic extract of stem(48.13 mg /g).The result indicates the antioxidant and antibacterial activity potential of *A. mexicana*.

Key words: *Argemone mexicana,* DPPH assay, antioxidant activity, and antibacterial activity.

Citation: Pavithra, S., Dr. Sekar, T. and Sujatha, S., 2018. "Anti-bacterial and free-radical scavenging activity plant leaf, stem and root of argemone mexicana. (l.)" *International Journal of Current Research in Life Sciences*, 7, (04), 1836-1841.

INTRODUCTION

Medicinal plants are source of diverse nutrients and bioactive compounds that plays a significant main role in therapeutically application. Scientific interest in medicinal plants has increased due to phytochemicals which provide unlimited opportunities for new drug discoveries (Cosa *et al.,* 2006).The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidant defence mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in re-antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad *et al.,* 2006). Among the numerous naturally occurring antioxidants, ascorbic acid, carotenoids and phenolic compounds are more effective (Duh *et al.,* 1999). The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Cao *et al.,* 1996). On continuation of our experimental work for the search of antioxidant activity of medicinal plants, we studied extracts of six medicinal plants. The free radical scavenging activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) was evaluated during the course of work.

Corresponding author: **Pavithra, S.,**
Department of Botany, Bharathiar University, Coimbatore, Tamilnadu – 641 046, India.

The ascorbic acid, carotenoids and total phenol contents with antioxidant activity were also determined. The assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants. As such, production offree radicalsand otherreactive oxygen species in the human body by numerous physiological and bio-chemical processes is reported (Halliwell, 1994). Antioxidants offer resistance against oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent the disease progression (Braughler *et al.,* 1986). The most commonly used synthetic antioxidants at present are ButylatedHydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), Propyl Gallate and Tert-Butylhydroquinone. However, they are suspected of being responsible for liver damage and acting as carcinogens in laboratory animals (Anagnostopoulou *et al.,* 2006). The search for new products with antioxidative properties and fewer side effects is very active domain of research. Therefore, the development and utilization of more effective antioxidants of natural origin is desirable (Sakagami *et al.,* 1991). The plant *A.mexicana*cures leprosy, skin diseases, inflammation and bilious fevers. Roots are useful in guineaworm infestation and ects. The latex is useful in dropsy, jaundice, skin diseases, leprosy, blisters, conjunctivitis, inflammation, burning sensation and malarial fever. The oil is useful in indolent ulcers, wounds, leprosy and skin diseases, constipation, flatulences, colic and rheumatalgia.

In Homeopathic system of medicine the drug prepared from this herb is used to treat the problem caused by tape worm. (Nacoulma, 1996; Rajvaidhya *et al.,* 2012).

MATERIALS AND METHODS

Plant material

The leaf, stem and root parts of *A.mexicana*were collected by S. Pavithra from Maruthamalai tail of Western Ghats, during November 2015. The collected plant material was identified and authenticated by Botanical survey of India, Southern Circle, Coimbatore (NO.BSI/SRC/5/23/2015/Tech)and the voucher specimen has been deposited in Bharathiar University Herbarium, Department of Botany, and Coimbatore. The collected fresh plant for leaves, stem, and root were cleaned thoroughly with running tape water to remove dust and shade dried for a week at room temperature. The powers were in airtight container.

Plant extracts preparation

The powdered plant material was extracted in Soxhlet extractor successively with petroleum ether, chloroform, acetone and methanol. Each time before extracting with the next solvent, the thimble was dried in hot air oven below 40 ºC. The different solvent extracts were concentrated by rotary vacuum evaporator and then air dried. The dried extract obtained with each solvent was weighed. The percentage yield was expressed in terms of air dried weight of plant material.

Quantification of total phenolics, tannins and flavonoids
Quantification of total phenolics and tannin

The total phenol content was determined according to the method described by (Makkar, 2003). 100 µL aliquots for plants extracts (5mg/ mL) were taken in the test tubes and made up to the volume of 1mL with distilled water. Then 0.5mL ofFolin - Ciocalteu reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against blank. Reaction mixture without plant extract was taken as blank. The analysis was performed in triplicate and the results were expressed as Gallic acid equivalents. Using the same extract the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP) 1mg of PVPP was weighed into a 2 mL eppendorf tube and to this 900 µL distilled water and then 750 µL of the sample extracts were added. The content was vortexes and kept in the test tube at 4 ºC for 4hrs. Then the sample was centrifuged at 4000 for 10 minutes at room temperature and the supernatant was collected. This supernatant has only simple phenolics other than the tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured and expressed as the content of non-tannin phenolics. From the above results, the tannin content of the sample was calculated as follows:

Tannin (%) = Total phenolics (%) - Non tanninphenolics (%)

Quantification of total flavonoids

The flavonoid contents of all the extracts were quantified as it act as a major antioxidants in plants reducing oxidative stress. Estimated as per described by (Zhishen *et al.,* 1999).

Initially 150 µL of all the plant extracts were taken in different test tubes. To each extracts 2 mL of distilled water was added. Then 150 µL of $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 minutes. After incubation 150 µL of $AlCl_3$ (10%) was added to all the test tubes. The test tubes were incubated for 6 minutes at room temperature. Then 2 mL of NaOH was added to all the test tubes which were made up to 5 mL using distilled water. The contents in all the test tubes were vortexes well and they were allowed to stand for 15 minutes at room temperature. The pink colour developed due to the presence of flavonoids was read spectrophotometrically at 510 nm. The amount of flavonoids was calculated as rutin equivalents.

In vitro antioxidant studies

Radical scavenging activity using DPPH method

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$), according to the method of (Prieto *et al.,* 1999). Plants extracts at various concentrations (20 - 100 µL) was added to 5 mL of 0.1 m Methonolic solution of $DPPH^{\cdot}$ and allowed to stand for 20 min at 27 ºC. The absorbance of the sample was measured at 517 nm. Methanol was served as blank and solution without extract served as control. The mixture of methanol, DPPH and standard (ascorbic acid) served as positive control. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula. More significantly the IC_{50} of the extracts were also calculated.

Phosphomolybdenum assay

The antioxidant power of the extracts has been assessed with the phosphomolybdenum reduction assay according to (14). The assay was based on the reduction of the extract and subsequent formation of a complex. 0.5 mL of extract combined with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was incubated at 95 ºC for 90 minutes. The absorbance was taken at 695 nm using spectrophotometer. The results were calculated in ascorbic acid equivalents.

Percentage of Phosphomolybdenum = (Control OD – Sample OD / Control OD) × 100

Assay of superoxide radical (O_2^{*-}) scavenging activity

The assay was based on the capacity of the sample extract to inhibit formazan formation by scavenging superoxide radicals generated in riboflavin- light-NBT system (Dinis *et al.,* 1994). Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 2.33µg riboflavin and 12 mM EDTA, and 11.55 g NBT. Reaction was started by illuminating the reaction mixture with of sample extracts (100 µL) for 90 seconds. Reaction mixture with extract kept in dark served as negative control while the mixture without extract was taken as blank. Immediately after illumination, the absorbance was measure at 590 nm. The activity was compared to ascorbic acid. The percentage inhibition of superoxide anion generation was calculated using the following formula:

Percentage of inhibition = (Control OD – Sample OD / Control OD) × 100

Metal chelating activity

Iron II chelating activity was measured by the inhibition of the formation of Iron-(II)-ferrozine complex after preincubation of the sample. The Fe^{+} was monitored by measuring the formation of ferrous iron –ferrozine complex against methanol blank at 562nm.The chelating of ferrous ions by various extracts in plant was estimated by the method of (Nile and Khobragade, 2009).The chelating of ferrous ions by various extracts of *A. mexicana* was estimated . Initially, about 100µl the extract samples were added to 50µl of 2 mM $Fecl_2$ solution. Then the reaction was initiated by the addition of 200µl of 5mM ferrozine and the test tubes were vortexes well and left standing at room temperature for 10 minutes. The reaction mixture containing deionized water in place of sample was considered as the negative control absorbance of the solution was then measured spectrophotometrically at 562 nm against the blank (deionized water). EDTA was against the standard metal chelating agent and the results were expressed as mg EDTA equivalents/g extract chelate the ferrous ion was calculated by, Percentage chelation=(1-(ABS sample/ABS control))×100Ec_{50} value (mg extract / mL) is the effective concentration at which ferrous ions were chelated 50% by the extract.

Antibacterial activity

Antibacterial activity of *A.mexicana* leaf methanol extract determined using well diffusion method. The bacteria were cultured in nutrient broth at room temperature and kept in orbital shaking incubator (remi,India) at 200 rpm for 2-3 days. The mullerhinton agar plates (bacteriaculture) were prepared and microbial strains were swabbed. After 5 mins the well (5mm size) was made by using gel puncher and different concentrations (25µg, 50µg, 75µg and 100µg/ml) of the sample was added in the well. The positive control (10µg/ml) (tetracycline) was prepared and poured into wells. The plants were incubated at 37˚C for 24h (bacteria). After incubation the antibacterial activity was assessed. Each screening test was performed with three replicates and t the mean values are recorded.

RESULTS AND DISCUSSION

Estimation of total phenolic and tannins

The phenolic, tannin and flavonoid content of *A. mexicana*were estimated shows the table 1. Leafmethanolic extract has maximumphenolic content and was found to be 49.93 ±5.30mg GAE/100g of extract, when compared to other extract. Tannin content determination of plant extract showed that the leafmethanolic extract possess the higher tannin content (5.32 ±0.46 mg GAE/100g) of extract. The estimation of total flavonoids content in leaves, stems and roots revealed and the methanol leaf extract of possess the maximum (14.76 ± 0.65mg RE /100g) of extract. Phenolic and flavonoids are common groups of polyphenolic compounds and have important roles in stabilizing lipid per oxidation due to their anti - oxidative activities. Many studies have indicated that the antioxidant capacities of flavonoids are due to the number and position of hydroxyl groups in their structures (Hau *et al.,* 2009). These studies strongly support that *A. mexicana*un doubtedly can have antioxidant another medicinal from oxidative damage by possessing antioxidants such as polyphenolics compounds.

Antioxidant assays

DPPH radical scavenging activity

The free radical-scavenging activities of leaf, stem and root of the plants *A.mexicana*extract investigated antioxidant activity of DPPH and the results are shown in figure 1. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. The colour change from purple to yellow is visually evident. A lower value of IC_{50} (inhibitory concentration at 50%) indicates a higher antioxidant activity. Generally, the acetone and methanol extracts of all the parts showed significant reduction of DPPH radical. However the highest was free *A.mexicana r*adical scavenging activity was exerted by methanol extract of leaf, stem and root (IC_{50} value were 10.25µg/ml;11.22µg/ml; 11.89µg/ml).

Table: 1. Total phenolic, tannins and flavonoid content of leaf and stem extract of *A.mexicana.*

S.No	Plant material	Extract	Total phenol (GAE mg/100g)	Tannin (GAE mg/100g)	Flavonoid (RE mg/100g)
1.		Pet ether	28.38± 2.27	3.12 ±0.46	9.16±1.10
2.		Chloroform	22.26± 3.17	2.31 ±0.22	12.42±0.65
3.	*A.m.*leaves	Acetone	32.26±3.17	5.01±0.44	13.60±1.31
4.		Methanol	49.93 ± 5.30*	5.32±0.46*	14.76±0.65*
1.		Pet ether	20.14 ± 1.02	2.31 ±0.22	10.49±1.65
2.		Chloroform	21.14± 0.22	3.05 ±0.33	8.04 ± 1.25
3.		Acetone	37.63± 5.62	4.25 ±0.46	11.09±1.51
4.	*A.m*. stem	Methanol	39.81 ± 2.73*	4.88±0.58*	12.03±1.24*
1.		Pet ether	16.78 ± 1.23	0.12±0.71	10.71±2.37
2.		Chloroform	17.9 ± 2.30	1.25 ±0.33	10.93±1.00
3.		Acetone	38.69 ± 2.62	4.23 ±0.67	11.02±0.65
4.	*A.m*. root	Methanol	34.22± 1.25*	4.55 ±0.33*	11.12±1.36*

Values are mean of replicate determination (n=3)±standard deviation. GAE-Gallic acid equivalence, RE-Rutin equivalence.

Table 2. Antibacterial activity of *A. mexicana* leaf methanol extract

Organisms	Control (100µg/ml)	Zone of inhibition in diameter(mm)			
		25(µg/ml)	50(µg/ml)	75(µg/ml)	100(µg/ml)
staphylococcus pyogens	35±0.5	28±0.05	30±1.03	33±0.05	34±0.07
staphylococcus aureus	38±0.5	25±0.01	29±0.03	32±0.07	36±0.03
E.coli	36±0.4	21±0.05	25±0.05	32±0.03	34±0.01
Pseudomonas Aeruginosa	33±0.1	26±0.5	25±0.03	30±1.02	31±0.06
klebsiella pneumonia	25±1.03	15±0.03	17±0.04	19±0.6	22±0.03

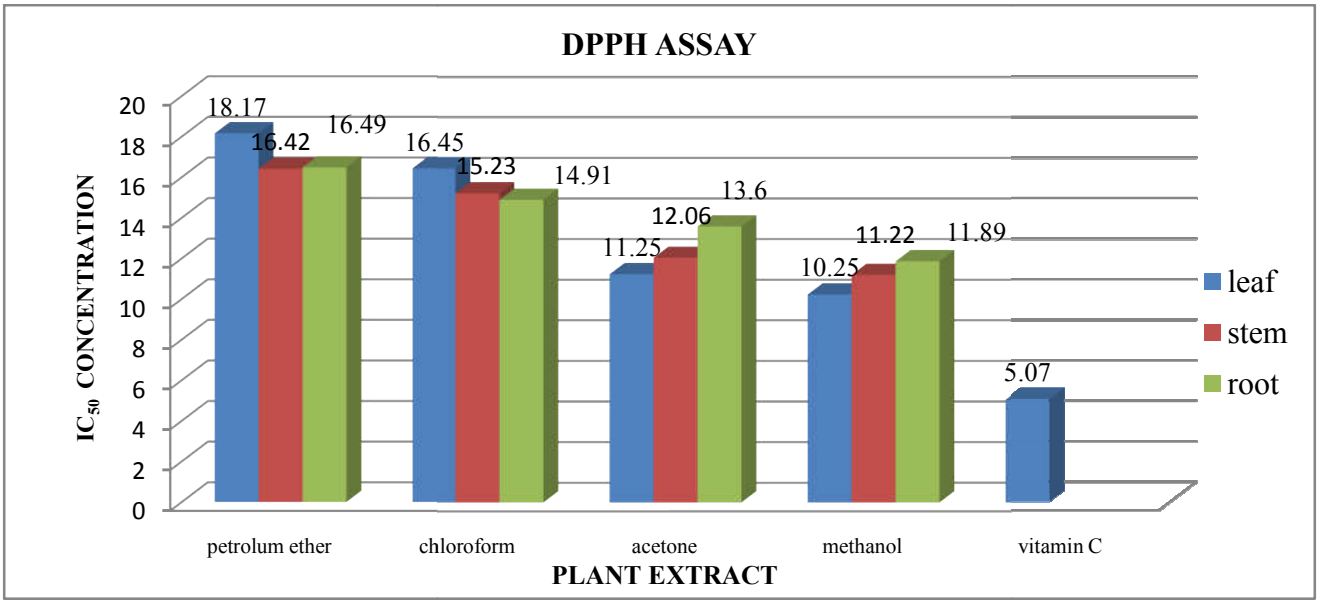


Figure 1. DPPH scavenging activity of *Aregmone mexicana* leaf, stem and root extract

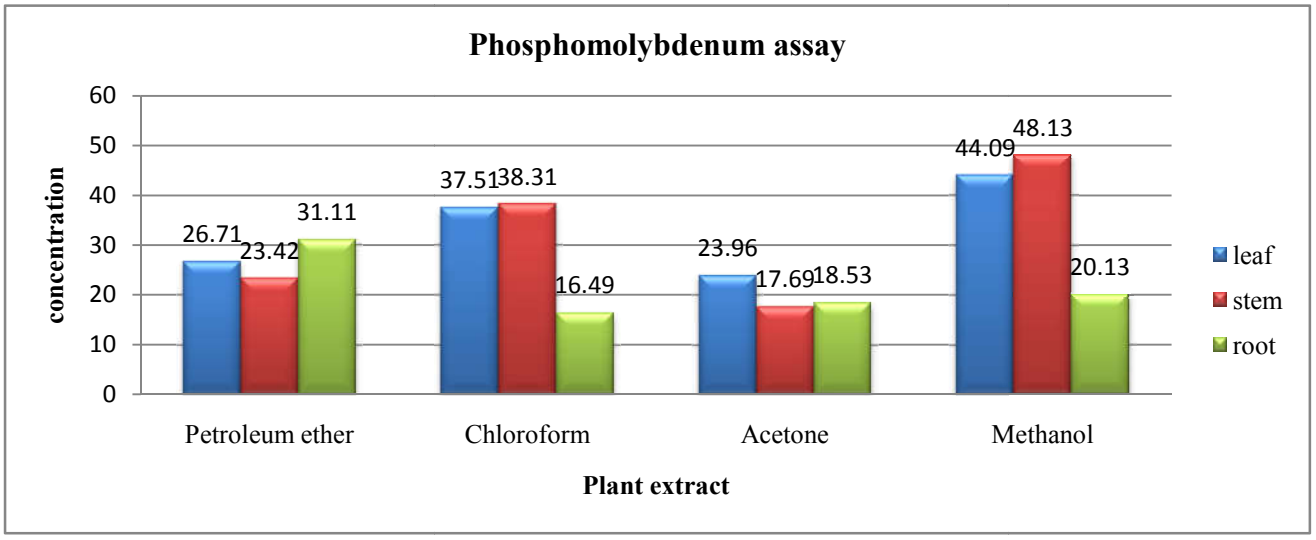


Figure 2. Phosphomolybdenum assay leaf, stem, and roots extracts of *A.mexicana*

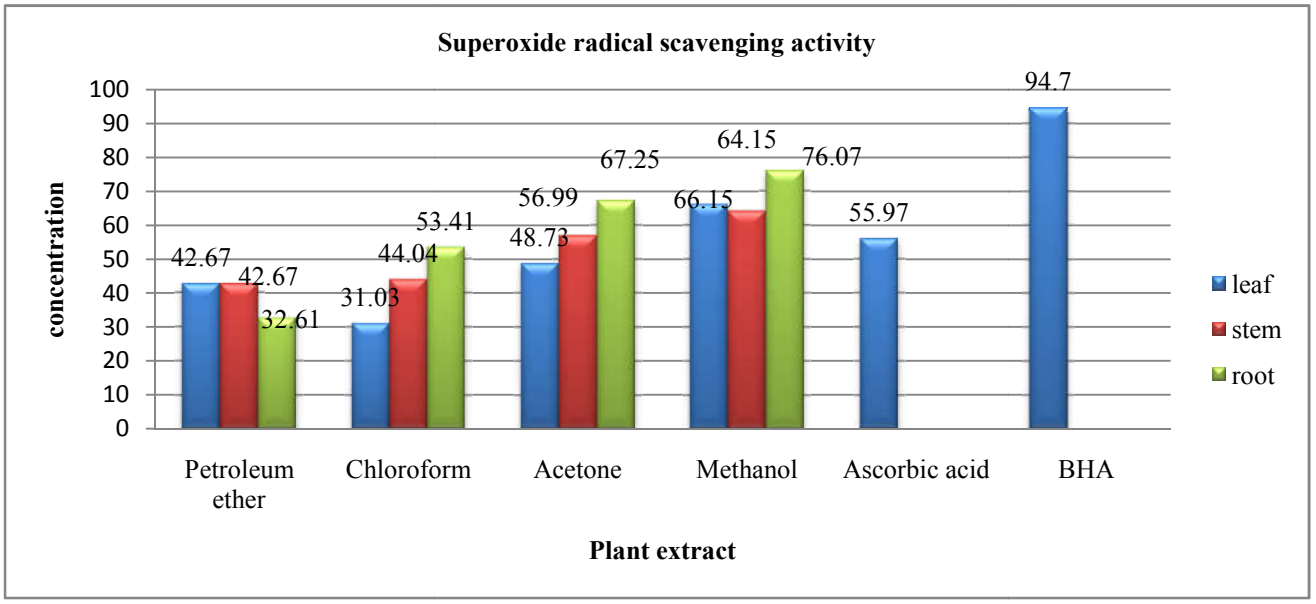
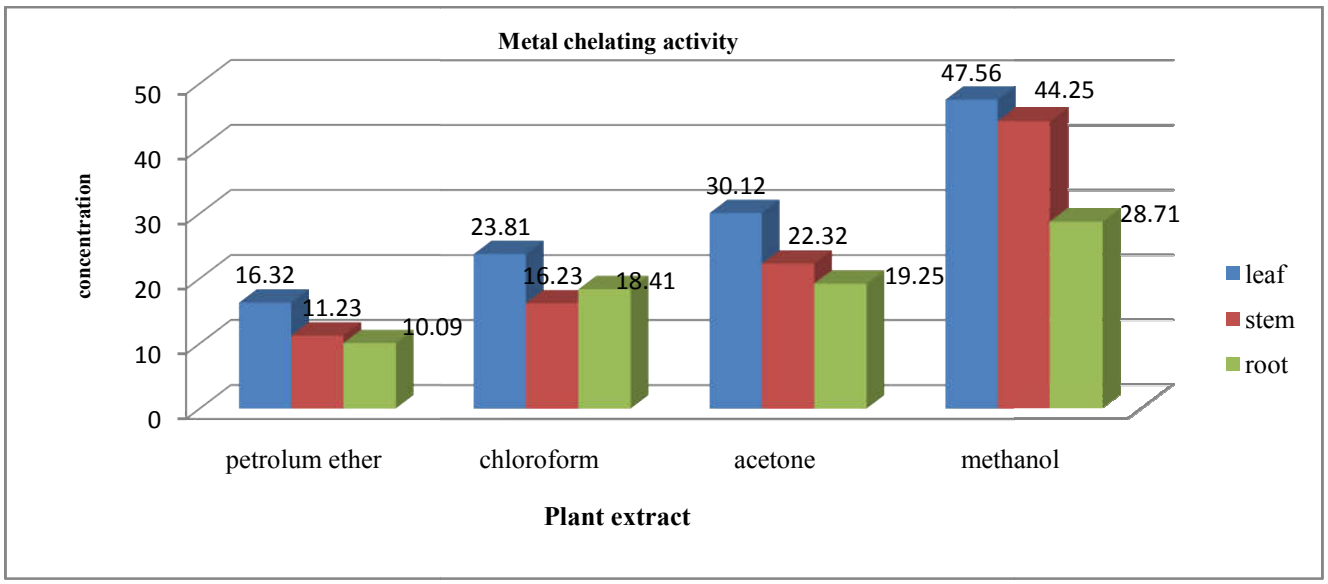
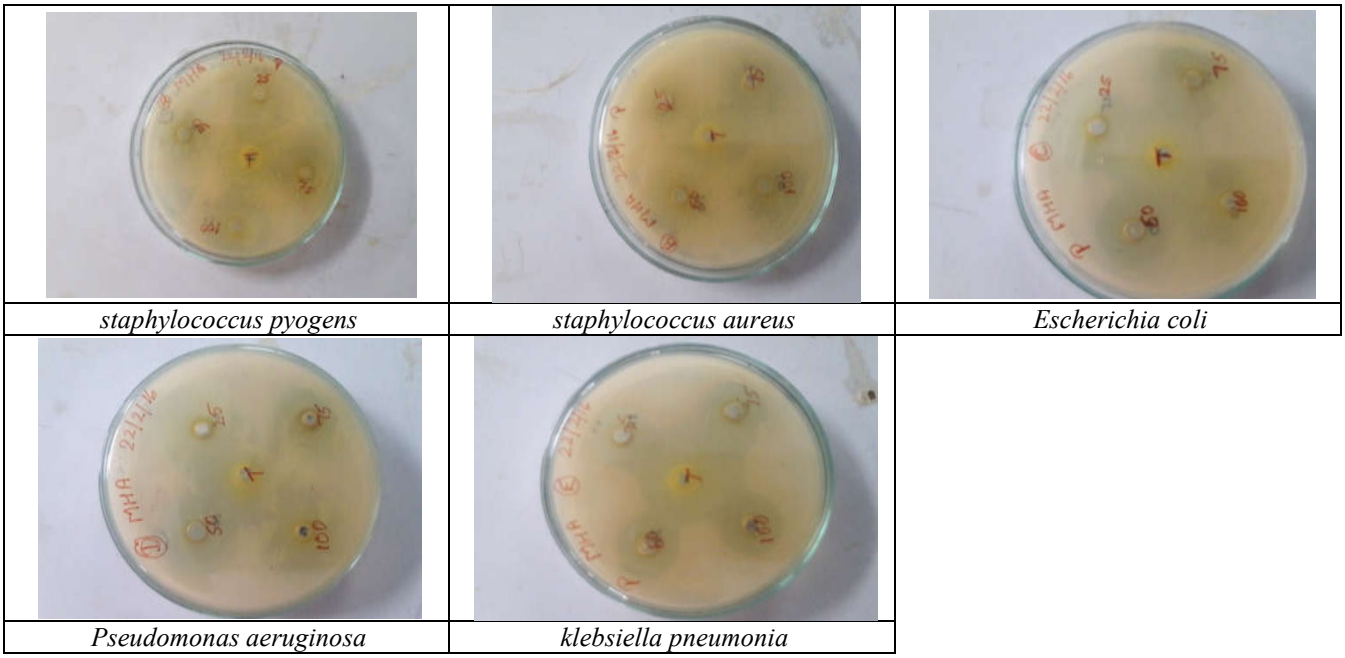


Figure 3. Superoxide radical scavenging activity of *A.mexicana* leaf, stem, and root extracts



Plates for Anti-bacterial activity of*A.mexicana*leaf methanolic extract



staphylococcus pyogens	*staphylococcus aureus*	*Escherichia coli*
Pseudomonas aeruginosa	*klebsiella pneumonia*	

Phosphomolybdenumassay

The antioxidant activity of phosphomolybdenum assay analysed and the results are show figure 2. The phosphomolybdenum reduction assay was based on the reduction of Mo(VI) to Mo(V) in presence of antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acidic pH and at higher temperature. Among *A. Mexicana* stem showed higher activity in compared to other solvents extracts of leaf and root. Methanol extract of stem (48.13 mg/g extract) have highest phosphomolybdenum reduction compared to other solvent extracts.

Superoxide radical scavenging activity: The results of superoxide anion scavenging of different extracts of leaf stem and root were analysed. *A.mexicana* root methanolic extract was higher (76.07% ;), Then the plant stemand leaf methanol extract (64.15%; 66.15%). *A.mexicana* low significant activity was shown stem as compared to leaf and stem. These results were compared with natural (Ascorbic acid) and synthetics (BHT) antioxidants.

Metal chelating activity

Ability of antioxidants to form insoluble metal complexes with ferrous ion or to generate stearic hindrance that prevent interaction between metal and lipid is evaluated using the ion chelating capacity (Elmastas *et al.,* 2005). The activity is measured by monitoring the decrease in absorbance of lead Ferricferrozine complex as antioxidants compete with ferrozine in chelating ferrous ion (Soler-Rivas *et al.,* 2000).The Fe^{+} chelating capacity of different solvent extracts of leaf and stem of *A.mexicana* were analyzed, inplantmaximum activity were observed for the methanolic extract (47.56 mg EDTAE/100g; 44.25 mg EDTAE/100g) extract as compared to other extract.

Antibacterial activity

The antibacterial activity of the *A.mexicana* methanol extract. The zone of inhibition was maximum at the concentration (100µg/ml) of given sample the highest zone of inhibition was observed in *staphylococcus aureus* (36±0.03mm) at concentration of 100µg/ml. Similarly the lowest zone of

inhibition was observed in *klebsiella pneumonia (*22±0.03mm) at concentration of 25µg/ml. The results confirm that the given sample shows excellent antibacterial activity.

Statistical analysis

Total phenolic, tannin and total flavonoids contents analyses were carried out in triplicates, the results of the contents were performed from the averages of all samples reading Mean ± SD used Excel 2003. The antioxidant data were statistically analyzed using one way ANOVA followed by Duncan's test for antioxidant studies Mean value were considered statistically significant when $P<0.05$.

Conclusion

Numerous phytochemical screening studies have been carried out in different parts of the world using antioxidant studies. In the present study, *A .mexicana* extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. In addition to this, the results of the antioxidant profile can be used as pharmacological tool for the medicinal purposes of this plant.

Acknowledgements

The authors would like to thank Dr.T.Sekar, Assistant Professor, Department of Botany, Bharathiar University, Coimbatore, for providing technical assistance during the research work.

REFERENCES

Anagnostopoulou, MA., Kefalas, P., Papageorgiou, VP. and Boskou, D. 2006. Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis). *Food Chem.,* 94: 19-25.

Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase: Improved assay applicable to acrylaminde gels. *Analytical Biochemistry*., 44: 276-277.

Biswas, A., Bhattacharya, A., Chattopadhyay, A., Chakravarty A. and Pal, S. 2011. Antioxidants and Antioxidant Activity in Green Pungent Peppers. *International Journal of Vegetable Science,* 17: 224-

Braughler, JM., Duncan, LA. and Chase, RL. 1986. The involvement of iron in lipid peroxidation. Importance of ferric to ferrous ratios in initiation. *J Biol Chem.,* 261: 10282-10289.

Cao, G., Sofic, ER. and Prior, RL. 1996. Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem*., 44: 3426-3431.

Cosa, P., Vlietinck, A.J., Berghe, D.V. and Maes, L. 2006. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept' *J Ethnopharmacol.,* 106:290–302.

Dinis, TC., Madeira, VM., Almeida, LM. 1994. Action of phenolic derivatives (acetaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch Bio chem Bio phys.,* 315:161-9.

Duh, PD., Tu, YY. and Yen, GC. 1999. Antioxidants activity of aqueous extract of Harnjyur (Chrysanthemum morifolium Ramat). *Lebens mwiss Technol*., 32: 269-277.

Elmastas, *et al.,* 2005M. Elmastas, I. Gulcin, L. Ozturk, I. GokceInvestigationof antioxidant properties of spearmint (Menthaspicata L.) *Asian Journal of Chemistry*, 17 (1) (2005), pp. 137-148

Halliwell, B. 1994. Free radicals, antioxidants, and human disease: curiosity, cause, or Consequence. *Lancet,* 344:721–724.

Hau, D.K.P., Gambari, R. and Wong, R.S.M. 2009. Phyllanthusurinaria extract attenuates acetaminophen induced hepatotoxicity: Involvement of cytochrome P450 CYP2E1. *Phytomedicine,* 16: 751–760

Makkar, H.P.S. 2003.Quantification of tannins in tree and shrub foliage: A laboratory Mannual. Dondrecht. The Netherlands : Kluwer academic publishers.

Nacoulma, O. 1996. Plantesmédicinaleset pratiques médicalestraditionnelles au Burkina Faso .Cas du plateau central. TOME II.Thèsed'Etat. UnivOuaga, 332p.

Nile, SH. and Khobragade, CN. 2009. Determination of nutritive value and mineral elements of some important medicinal plants from western part of India. *J Med Plants*, 8: 79–88.

Pourmorad, F., Hosseinimehr, SJ. and Shahabimajd, N. 2006. Antioxidant activity, phenols, flavanoid contents of selected Iranian medicinal plants. *S. Afr. J. Biotechnology*, 5: 1142-1145.

Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. *Analytical Biochemistry,* 269, 337-341.

Rajvaidhya, S., Nagori, B.P., Singh, G.K., Dubey, B.K., Desai, P. and Jain, S. 2012. A review on Argemonemexicana Linn.an Indian medicinal plant. *International Journal of pharmaceutical Sciences and Research*, 3(8): 2494-2501.

Sakagami, H1., Aoki, T., Simpson, A. and Tanuma, S. 1991. Induction of immunopotentiation activity by a protein-bound polysaccharide, PSK (review). *Anticancer Res.,* 11: 993-999.

Soler-Rivas C., J.C. Espin, H.J. Wichers An easy and fast test to compare total free radical scavenger capacity of foodstuffs. *Phytochem. Anal.,* 11 (2000), pp. 330-338

Zhishen, J., Mengecheng, T. and anJianming, W. 1999. The determination of flavonoids contents on mulberry and their scavenging effects on superoxide radical. *Food chemistry*, 64: 555-559.

****