ejpmr, 2018,5(9), 283-292

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

IN VITRO ANTIOXIDANT ACTIVITY OF *PETIVERIA ALLIACEA* L., (PHYTOLACCACEAE) AND *BELLOPERONE PLUMBAGINIFOLIA* (ACANTHACEAE)

[1]G. Sathiya Balan, [2]Dr.V.R.Mohan, [3]Dr.S.Muthukumarasamy, [4]K.Muthukrishnan

[1]Assistant Professor, Department of Pharmacognosy, Madurai medical college, Madurai, Tamilnadu, India.

[2]Associate Professor and Head, Ethanopharmacology unit, V.O.C College, Tuticorin, Tamilnadu, India.

[3]Associate Professor and Head, Department of Botany, Sri K.G.S Arts College, Srivaikundam, Tamilnadu, India.

[4]Department of Pharmacognosy, Madurai medical college, Madurai, Tamilnadu, India.

***Corresponding Author: G. Sathiya Balan**

Assistant Professor, Department of Pharmacognosy, Madurai medical college, Madurai,

Article Received on 12/07/2018 **Article Revised on 01/08/2018** **Article Accepted on 22/08/2018**

ABSTRACT

Antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Petiveria alliacea* L.,) (phytolaccaceae and *Belloperone plumbaginifolia* (acanthaceae) have been tested using various antioxidant model systems viz., DPPH, hydroxyl, superoxide and ABTS, reducing power assay. Methanol and ethanol extracts of the of *Petiveria alliacea* L., (phytolaccaceae) and *Belloperone plumbaginifolia* (acanthaceae) showed strong DPPH and hydroxyl radical scavenging activity where as ethanol and methanol extract showed strong superoxide and ABTS radical cation scavenging activity respectively. The IC50 values in all models viz., DPPH, hydroxyl, superoxide, reducing power and ABTS radical cation scavenging activity of methanol and ethanol extract of *Petiveria alliacea* L., (phytolaccaceae) and *Belloperone plumbaginifolia* (acanthaceae) were found different concentration. This study indicates significant free radical scavenging potential of the *Petiveria alliacea* L., (phytolaccaceae) and *Belloperone plumbaginifolia* (acanthaceae) which can be exploited for the treatment of various free radical mediated diseases.

KEYWORDS: *Petiveria alliacea.L. Belloperone plumbaginifolia*, flavonoid, anti-oxidant, ABTS, reducing powder.

INTRODUCTION

Antioxidants are important in the prevention of human diseases. Naturally occurring antioxidants in leafy vegetables and seeds, such as ascorbic acid, vitamin E and phenolic compounds, possess the ability to reduce the oxidative damage associated with many diseases, inducing cancer, cardiovascular disease, cataracts, artherosclerosis, diabetes, arthritis, immune deficiency diseases and aging (Basniwal *et al*., 2009). Antioxidant means 'Against oxidation' which work to protect lipids from peroxidation by free radicals. Oxidants can damage cells and food substance by starting chain reactions such as lipid peroxidation or by oxidizing DNA or Proteins (Jenecius *et al*., 2012). Bioactive compounds derived from the plant kingdom have been successfully used to reduce lipid oxidation in food industry products (Dolai *et al*., 2012; Bernatoniene *et al*., 2011). Organisms have also evolved complex mechanisms via antioxidants metabolites and enzymes met work in concert to prevent oxidative damage (Sharmila Jose and Radhamani., 2012). These antioxidants are capable of inhibiting the oxidation of biomolecules by removing free radical intermediates and inhibiting other oxidation reactions. Antioxidants could also interrupt peroxidation by donating hydrogen atom rapidly to a lipid radical, forming a new radical, more stable than the initial one oxidative stress occurs when there are low levels of antioxidants or inhibition of the antioxidant enzymes resulting in cell damage or cell death(Awah *et al*., 2012). Several commercially available synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) are currently in use but their possible toxic properties for human health and environment are inevitable (Harini *et al*., 2012). Hence the development of alternative antioxidants from natural origin is the need of the hour. Therefore, it is important to assess antioxidant activity of the plants used in the herbal medicine either to elucidate the mechanism of their pharmacological action or to provide information on antioxidant activity of these herbal plants (Molan *et al*., 2012) Mangroves are biochemically unique, producing a wide array of novel natural products. Substances in mangroves have long been used in folk medicine to treat

diseases. Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal, antiplasmodial and hepatoprotective activities (Ravikumar and Gnanadesigan., 2011; Gnanadesigan *et al*., 2011). They provide a rich source of steroids, triterpenes, saponins, flavanoids, alkaloids and tannins (Kanchanapoom *et al*., 2001; Subasree *et al.*, 2010; Xu *et al*., 2004; Ravikumar *et al*., 2010). But, the studies related with *in vitro* antioxidant activity from mangrove plants are too limited. Therefore the main objective of the study is to screen *in vitro* antioxidant activity of the different solvent extracts of *Petiveria alliacea* L., (phytolaccaceae) and *Belloperone plumbaginifolia* (acanthaceae).

MATERIALS AND METHODS

Collection of plant material

The whole plant of *Petiveria alliacea* L. and *Beloperone plumbaginifolia* (Jacq.) Nees. were collected from four different sites, around a radius of 1 km, at Pechiparai, Kanyakumari District, Tamil Nadu (Area map). The plant sample was identified with the help of local flora and authenticated by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen of collected plants was deposited in the Ethnopharmacology Unit, PG & Research Department of Botany, V.O. Chidambaram College, Thoothukudi District, Tamil Nadu, India.

Preparation of extract

100 g of the coarse powder of the whole plant of *Petiveria alliacea* and *Beloperone plumbaginifolia* were extracted successively with 250 ml of petroleum ether, benzene, ethyl acetate, methanol and ethanol in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No. 41 filter paper separately and all the extracts were concentrated in a rotary evaporator. All the concentrated extracts were subjected to qualitative tests for the identification of various phytochemical constituents and used for *in vitro* antioxidant activity. The methanol extracts were subjected to quantitative test for the total phenolics and flavonoids. The ethanol extracts were used for the GC-MS analysis and pharmacological studies.

Estimation of total phenolics

Total phenolic content was estimated using Folin-Ciocalteau reagent based assay as previously described (McDonald *et al.*, 2001) with little modification. To 1 ml of each extract (100 µg/ml), 5 ml of Folin-Ciocalteau reagent (diluted ten- fold) and 4ml (75 g/L) of Na2CO3 were added. The mixture was allowed to stand at 20ºC for 30 minutes and the absorbance of the developed colour was recorded at 765 nm using UV-VIS spectrophotometer. 1 ml aliquots of 20, 40, 60, 80, 100 µg/ml methanolic gallic acid solutions were used as standard for calibration curve. The absorbance of solution was compared with gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100 g dry weight of extract).

Estimation of flavonoids

Total flavonoid content was determined according to Eom *et al*. (2007). An aliquot of 0.5 ml of samples were mixed with 0.1 ml of 10% aluminium chloride and 0.1 ml of potassium acetate (1M). In this mixture, 4.3 ml of 80% methanol was added to make 5 ml volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415 nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of Whole plant of methanol extract of *P. alliacea* and *B. plumbaginifolia* are given in Fig.1 and 2**.** The scavenging effect of ascorbic acid, the standard and various solvent extracts studied increased with the increase in the concentration. Among the solvent tested, whole plant methanol extracts of *P. alliacea* and *B. plumbaginifolia* exhibited the highest DPPH radical scavenging activity. At 800µg/ml concentration, the whole plant methanol extracts of *P. alliacea* and *B. plumbaginifolia* exhibited 131.16% and 136.93% DPPH radical scavenging activity respectively. IC50 values of whole plant methanol extracts of *P. alliacea* and *B. plumbaginifolia* on DPPH radical was found to be 37.12µg/ml and 34.18 µg/ml respectively and for for both the experimental plants the IC50 value was 28.13 µg/ml for ascorbic acid.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of whole plant petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *P. alliacea* and *B. plumbaginifolia* are given in Fig.3 and 4. The whole plant methanol extract of *B. plumbaginifolia* showed a very potent hydroxyl radical scavenging activity and the whole plant benzene exreact of *P. alliacea* showed the potent hydroxyl radical scavenging activity. At 800µg/ml concentration, *P. alliacea* and *B.plumbaginifolia* possessed 128.16% and 129.81% hydroxyl radical scavenging activity respectively. The quantity of *P. alliacea* and *B. plumbaginifolia* whole plant extracts required to produce 50% inhibition of hydroxyl radical was 33.14µg/ml and 33.18µg/ml respectively whereas 29.11µg/ml was needed for ascorbic acid to produce 50% inhibition.

Superoxide radical scavenging activity

All the extracts of *P. alliacea* and *B. plumbaginifolia* whole plants were subjected to be superoxide radical scavenging activity and the results are shown in Fig.5 and 6. The maximum superoxide radical scavenging activity, at 800µg/ml concentration, was exhibited by the whole plant benzene extracts of *P. alliacea* and *B. plumbaginifolia* (128.16% and 131.86% respectively). This scavenging activity was higher than that of the standard ascorbic acid (103.16%). IC50 values of whole plant benzene extracts of *P. alliacea* and *B. plumbaginifolia*, on superoxide radical lscavenging activity, were 27.31µg/ml and 24.16 µg/ml respectively

and the IC50 values for ascorbic acid were 30.16 µg/ml and 30.66 µg/ml respectivey.

ABTS radical scavenging activity

The different solvent extracts of *P. alliacea* and *B. plumbaginifolia* whole plants were subjected to be ABTS radical cation scavenging activity and the results are shown in Fig.7 and 8. The whole plant methanol extracts of *P. alliacea* and *B. plumbaginifolia* exhibited a potent ABTS radical cation scavenging activity in a concentration dependent manner. At 800µg/ml concentration, the whole plant methanol extracts of *P. alliacea* and *B. plumbaginifolia* possessed 131.16% and 131.93% ABTS radical cation scavenging activity respectively. IC50 values of methanol extracts of *P. alliacea* and *B. plumbaginifolia* on ABTS radical were 36.81µg/ml and 36.73 µg/ml respectively and for trolox, IC50 values of both the experimental plants were 30.66 µg/ml and 30.16 respectively.

Reducing power assay

Fig.9 and 10 showed the reducing abilities of different solvent extracts of *P. alliacea* and *B. plumbaginifolia* whole plants as compared to the standard ascorbic acid. Absorbance of the solution was increased with the concentration. A higher absorbance indicated a higher reducing power. Among the solvent tested, the whole plant methanol extracts of *P.alliacea* and *B.plumbaginifolia* exhibited higher reducing activity.

RESULTS AND DISCUSSION

Superoxide radicals, hydroxyl radicals, singlet oxygen, hydrogen peroxide and other such reactive oxygen species (ROS) are produced as by products during reaction or because of exogenous factors (Cerutti, 1991). In a biological process, a few ROS have a significant role such as producing energy, phagocytosis, regulating cell growth, intercellular signalling and also in the synthesis of vital biological compounds. But, these ROS can have adverse effects; also-attacking lipids in the cell membrane and DNA, spur oxidation which in turn lead to membrane damage like membrane lipid peroxidation and a reduction in membrane fluidity. They can also engender cancer causing DNA mutation (Pietta, 2000; Cerutti, 1994). A potent scavenger of these species may serve as a possible preventive intervention for free radical-mediated diseases (Ames *et al*., 1995). Recent studies showed that a number of plant products including polyphenolic substances (e.g. flavonoids and tannins) and various plant or herbal extracts exert antioxidant actions (Yokozawa *et al*., 1998; Marja *et al*., 1999; Gyamfi *et al*., 1999; Liu and Ng, 2000; Fejes *et al*., 2000).

It has been suggested that fruits, vegetables and plants contain a large variety of substances called phytochemicals which are present in plants and are the main source of antioxidant in the diet, which can decrease the potential stress caused by reactive oxygenspecies. The natural antioxidants may have free-radical scavengers, reducing agents, potential complexes of peroxidant metals, quenchers of singlet oxygen etc., (Ebadi, 2002). The antioxidants can obstruct with the oxidation process by reacting with free radicals (Gupta *et al*., 2004 a). As synthetic antioxidants are found to have adverse and cancer causing side effects, improved interests and efforts are needed to identify viable natural antioxidants that can be used in foods and medicinal substances (Kumaran and Karunakaran, 2007). Antioxidants derived from natural substances are multifaceted and their range and effects are also significant, offering immense scope for their exploitation (Shirwaikar *et al*., 2006).

Hence, in the present study, the whole plant extracts of *P. alliacea* and *B. plumbaginifolia* were investigated for their antioxidant activity using DPPH radical, hydroxyl radical, superoxide radical, ABTS radical cation scavenging activities and reducing power assay.

DPPH radical scavenging activity

DPPH is a relatively stable radical. DPPH reacts with suitable reducing agent. Depending on the number of electrons taken up, the electrons become paired off and solution loses colour stochiometrically (Blois, 1958). DPPH was used to determine the proton radical scavenging action of whole plant extracts of *P. alliacea* and *B.plumbaginifoilia*. It possesses a proton free radical and shows a typical absorbance at 517nm. From the results obtained in the present study, it may be postulated that whole plant extracts of *P. alliacea* and *B. plumbaginifoilia* reduce the radical to corresponding hydrazine when it reacts with hydrogen donors in antioxidant principles (Sanchez-Moreno, 2002). In the present study, among the solvents tested, the whole plant methanol extracts of *P. alliacea* and *B. plumbaginifoilia* exhibited the highest DPPH radical scavenging activity. Similar results were obtained with the methanol extracts of *Cassia fistula* (Ilavarasan *et al*., 2005), methanol extract of *C. auriculata* barks (Mishra *et al*., 2011) and methanol extracts of *C. occidentalis* leaves, stem and seed (Arya and Yadav, 2011). The results showed that the extracts with their proton donating ability could provide free radical inhibitors or scavengers, acting probably as primary antioxidants (Marxen *et al*., 2007).

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity is measured as the percentage of inhibition of hydroxyl radicals produced in the Fenton's reaction mixture (Braugghler *et al*., 1986) by revising the competition between deoxyribose and the extract for hydrogen radicals generated from Fe3+/ascorbate/EDTA/H2O2 systems. The hydroxyl radicals attack deoxyribose which eventually result in TBARS formation.

In the present study, whole plant methanol extract of *B. plumbaginifoilia* showed maximum hydroxyl radical scavenging activity when compared to standard ascorbic acid. Similar hydroxyl radical scavenging activity was

reported by (Ilavarasan *et al*., 2005) and Dheeraj *et al*., (2010) in *Cassia fistula* and *C. sophera* respectively. The results of the present study showed a close agreement with hyroxyl radical scavenging activity of *Sauropus bacciformis* (Jenecius *et al*., 2012), *Begonia malabarica*, *B. floccifera* (Kalpanadevi and Mohan 2012) and *Xanthosoma sagittifolium* (Nishanthini and Mohan, 2012).

Superoxide radical scavenging activity

Superoxides are produced from molecular oxygen due to oxidative enzymes (Sainani *et al*., 1997) of body as well via non enzymatic reaction such as autooxidation by catecholamines (Hemmani and Parihar, 1998). The scavenging action towards the superoxide radical (O2-) is measured in terms of inhibition of generation of O2-. The whole plant methanol extracts of *P. alliacea* and *B. plumbaginifoilia* possessed superoxide quenching ability. This results obtaines in the present study is in accordance with that of *Baccharis grisebachii* (Tapia *et al*., 2004) and *Calendula officinalis* (Preethi *et al*., 2006). Based on the results, it can be assumed that the extracts of *P. alliacea* and *B. plumbaginifoilia* scavenge superoxide radicals by combining with superoxide radical ions to form stable radicals, thus terminating the radical chain reaction (Wang *et al*., 2009).

ABTS radical cation scavenging activity

The inhibition of the absorbance of radical cation is based on the ABTS assay. ABTS has a characteristic long wave length absorption spectrum (Sreejayam and Rao 1996). The ABTS chemistry involves direct generation of ABTS radical mono cation with no association of any intermediary radical. It is a decolourization assay. Consequently, the radical cation is executed prior to addition of antioxidant test system. The results thus obtained imply the action of the extract by slowing down the ABTS radicals. This is because both inhibition and scavenging properties of antioxidants towards ABTS radicals and this has been reported in the past (Re *et al*., 1999). In the present study, whole plant methanol extracts of *P. alliacea* and *B. plumbaginifoilia* exhibited maximum ABTS radical cation scavenging activity when compared to standard trolox. Similar ABTS radical cation scavenging activities were reported by Tresina *et al.* (2012a,b) in *Eugenia singampattiana* and *E. floccosa*, (Kalpanadevi and Mohan 2012) in *Begonia malabarica* and *B. floccifera*, (Murugan and Mohan 2012) in *Dioscorea esculenta*.

Reducing power

Reducing power assay measures the electron-donating capacity of an antioxidant (Yen and Chen, 1995; Hinneburg *et al*., 2006). In this assay, the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. Presence of reducers cause the conversion of ferricyanide complex to the ferrous form used in this method may serve as a significant indicator of its antioxidant capacity (Yildirim *et al*., 2000; Amarowicz *et al*., 2004). The existence of reductones is the key of the reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom (Singh and Ranjini, 2004). The reduction of the ferricyanide complex to the ferrous form occurs due to the presence of reductants in the solution (Yen *et al*., 1993; Siddhuraju *et al*., 2002). Antioxidant components and their activity are highly dependent on extracting solvent and concentration of solvent, but they also vary within the samples. Many researchers have reported the relationship between phenolic content and antioxidant activity. In some studies, they found a correlation between the phenolic content and antioxidant activity (Velioglu *et al*., 1998: Yen *et al*., 1993: Kahkonen *et al*., 1999: Siddhuraju *et al*., 2002).

In the present study, higher absorption at higher concentration indicates the strong reducing power potential of the extracts. It is suggested that the extracts have high redox potential and can act as reducing agents.

Numerous studies have established a close link between total phenolic content and the antioxidant capability of the plant extract (Deighton *et al*., 2000: Vinson *et al*., 1998 and Velioglu *et al*., 1998). As the chemical composition and structure of active extract components are vital aspects that decide the effectiveness of naturally derived antioxidants, the antioxidant activity of any extract cannot be adequately explained considering only their phenolic content; it also requires their characterization (Heinonen *et al*., 1998).

For example, phenolic compounds that have artho and para-dihydroxylation or a hydroxyl and a methoxy group have been found to be more efficacious than simple phenols (Frankel *et al*., 1995 and Shahidi and Wanasundara, 1992). Flavonoids, phenolic acids, tannins and other such polyphenolic compounds are seen to exert critical influence on the antioxidant activities of fruits and vegetables with medicinal properties. Studies on phenol and phenolic compounds such as flavonoids have revealed to promote antioxidant activities; their influence on human nutrition and human health are significant (Kessler *et al*., 2003). But, the synergistic and additive functions of the phenolics in the extracts cannot be ignored. Fourteen compounds from *P. alliacea* and twelve compounds from *B. plumbaginifoilia* were isolated from the whole plant ethanol extracts by GC-MS analysis. Among the compounds isolated, 2-propenoic acid, 3-(4-methoxy phenyl)- ethyl ester, squalene and vitamin E showed antioxidant activity. This is the first report that envisages the antioxidant activities of different solvent extracts of whole plants of *P. alliacea* and *B. plumbaginifoilia*. Hence, the whole plants of *P. alliacea* and *B. plumbaginifoilia* could be a good source of antioxidant.

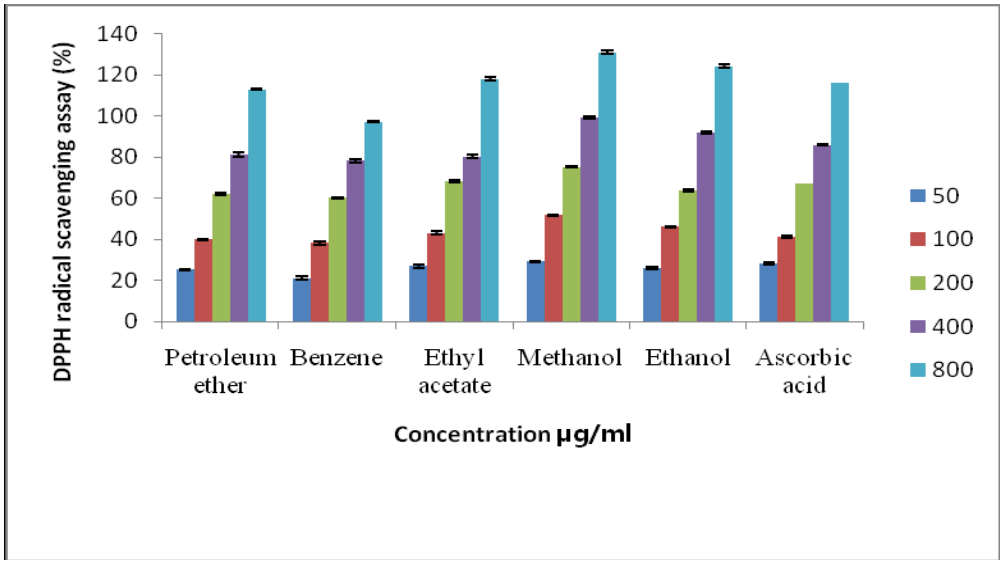

Fig 1: DPPH radical scavenging activity of different solvent extracts of *P. alliacea.*

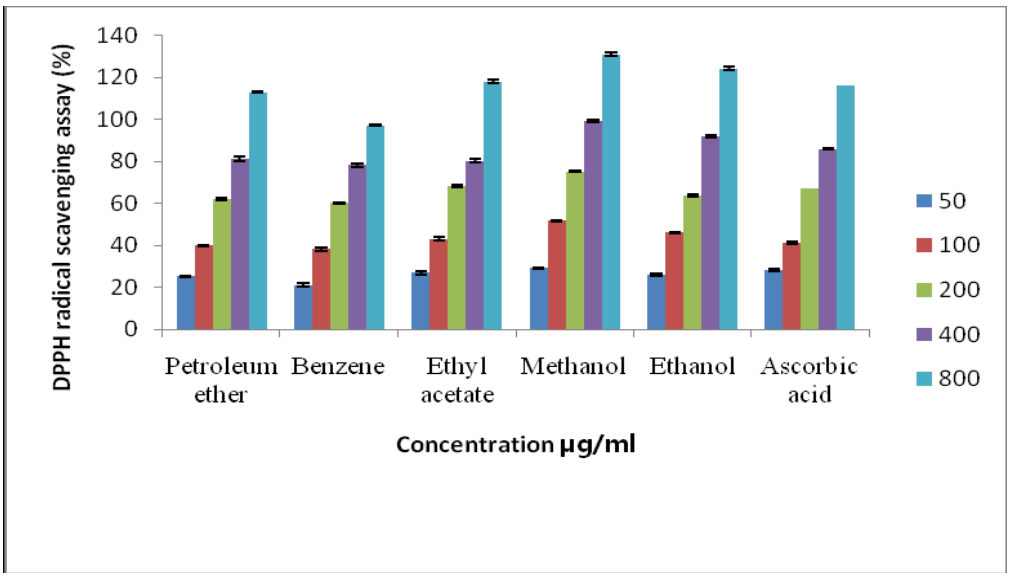

Fig.2: DPPH radical scavenging activity of different solvent extracts of *B.plumbaginifolia*

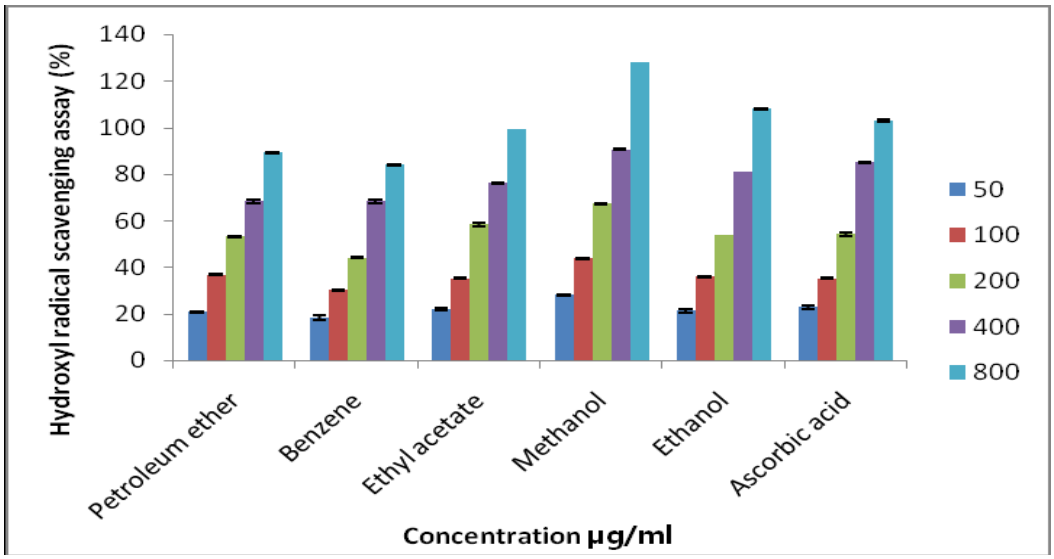

Fig.3: Hydroxyl radical scavenging activity of different solvent extracts of *P. alliacea.*

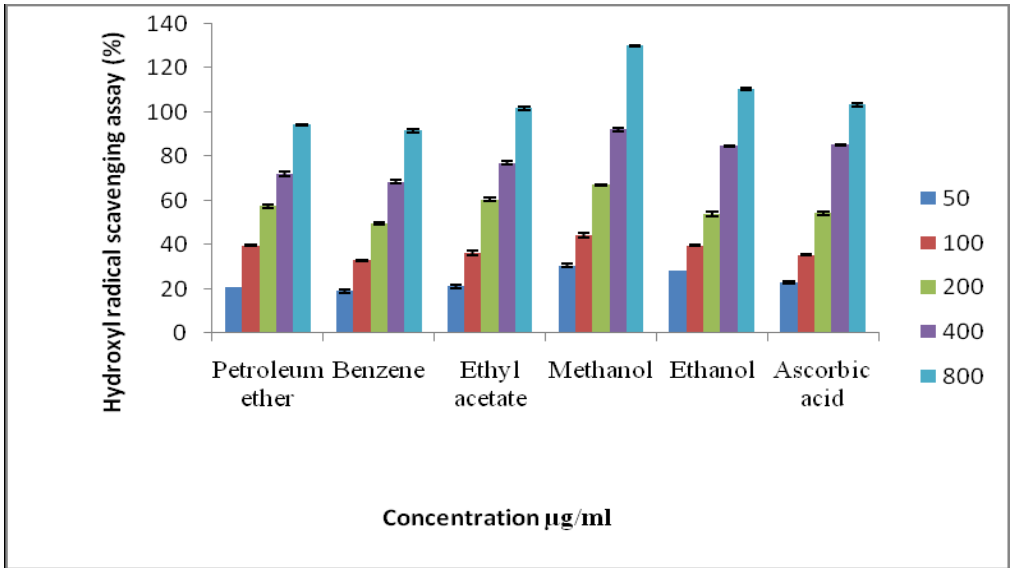

Fig.4: Hydroxyl radical scavenging activity of different solvent extracts of *B. plumbaginifolia.*

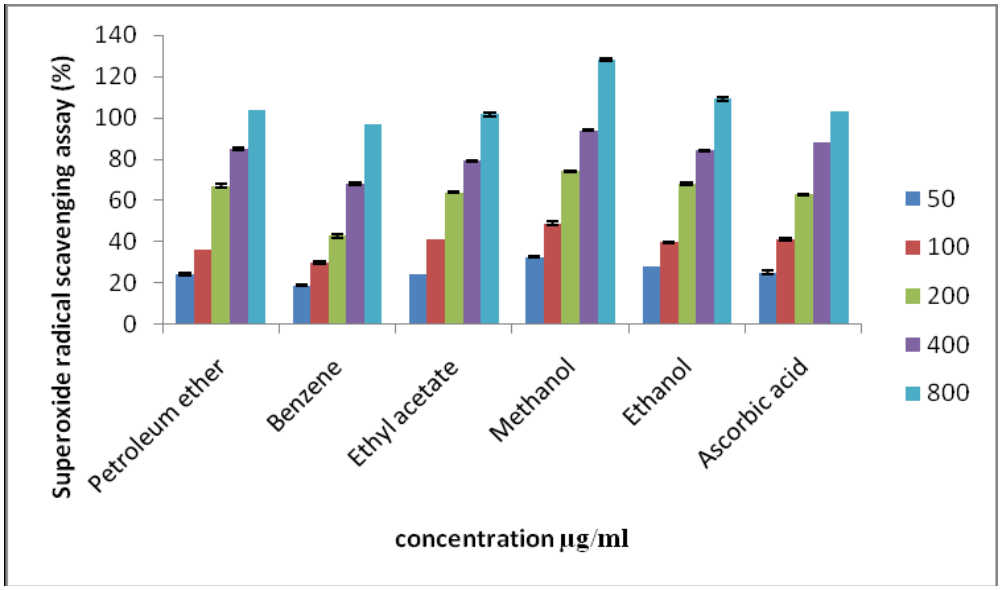

Fig.5: Superoxide radical scavenging activity of different solvent extracts of *P. alliacea.*

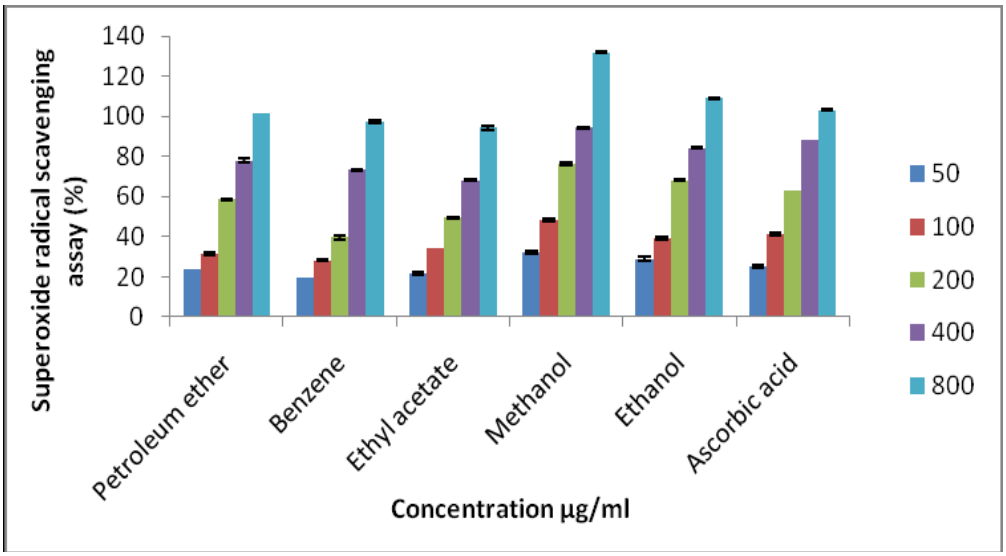

Fig.6: Superoxide radical scavenging activity of different solvent extracts of *B.plumbaginifolia.*

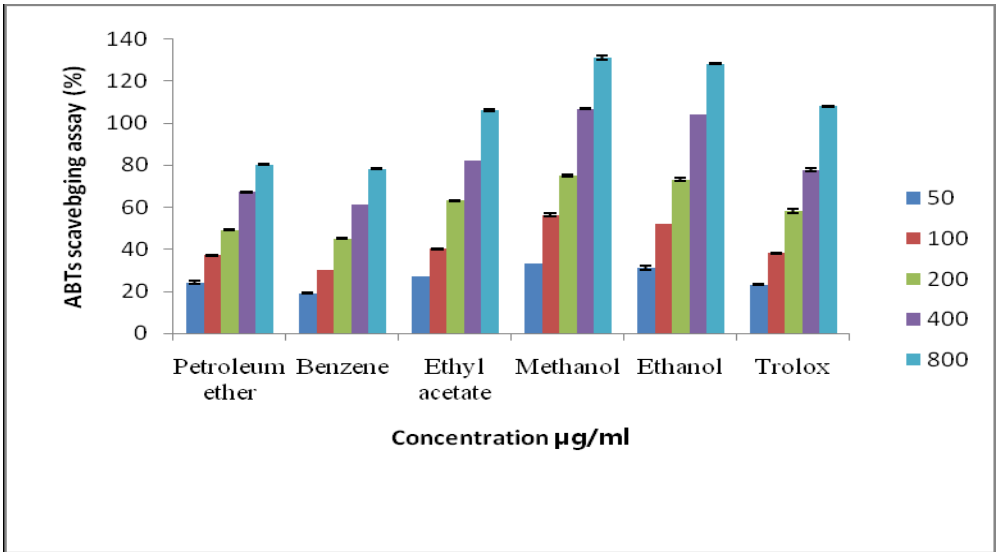

Fig.7: ABTs radical scavenging activity of different solvent extracts of *P. alliacea.*

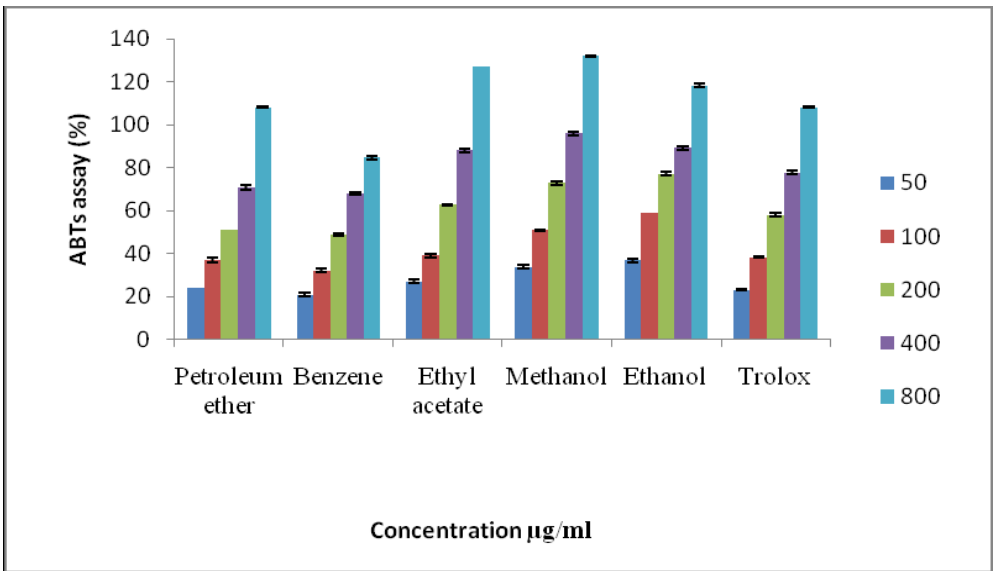

Fig.8: ABTs radical scavenging activity of different solvent extracts of *B. plumbaginifolia.*

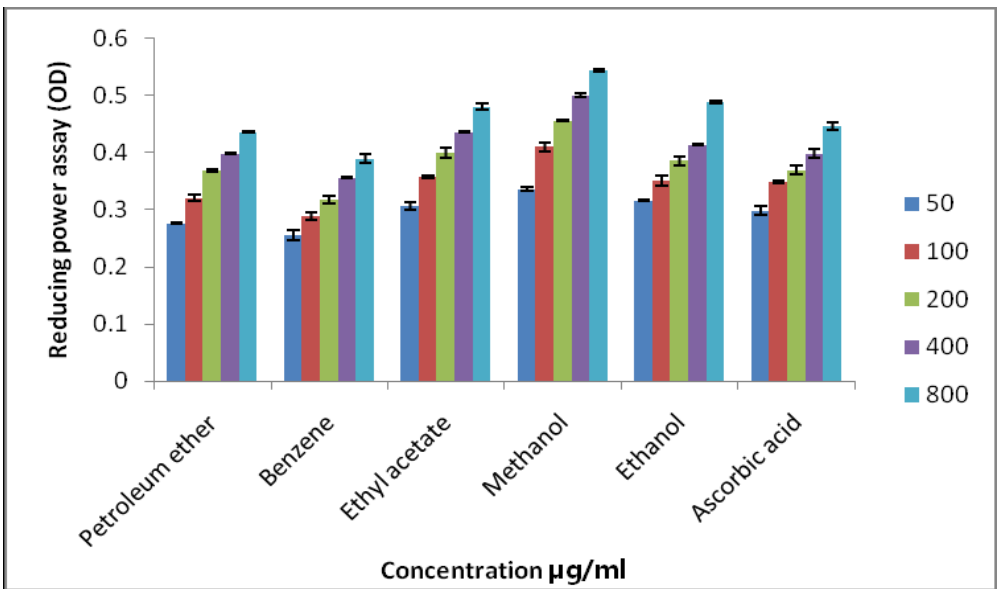

Fig.9: Reducing power assay of different solvent extracts of *P. alliacea.*

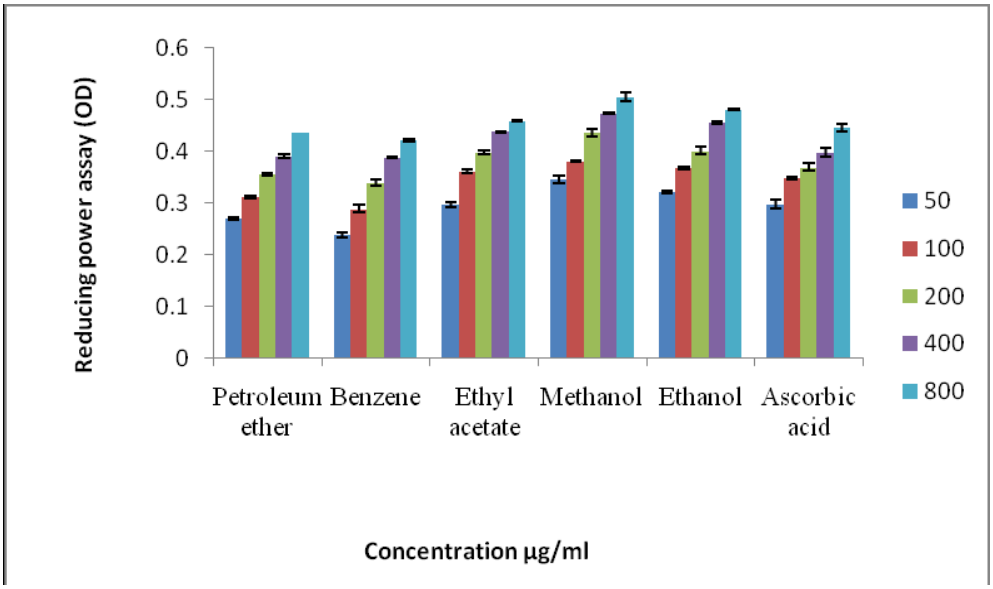

Fig.10: Reducing power assay of different solvent extracts of *B. plumbaginifolia.*

CONCLUSION

On the basis of results, in this study, it can be concluded that, all the extracts of *Petiveria alliacea L.*, (phytolaccaceae) and *Belloperone plumbaginifolia* (acanthaceae) is cabable of scavenging a wide range of free radicals. The extracts contain higher quantities of total phenolics and flavonoids, which exhibit antioxidant and free radical scavenging activity. *In vitro* assay systems confirm as *Petiveria alliacea L., and Belloperone plumbaginifolia* natural antioxidants but it is doubtful that specific compounds responsible for antioxidant activity. Further *in vivo* assessment is also needed to confirm antioxidant nature of *Petiveria alliacea* L., and *Belloperone plumbaginifolia*.

ACKNOWLEDGEMENT

The authors are thankful to Dr.R. Sampathraj, Honorary Director, Dr. Samsun Clinical Research Laboratory, Thiruppur for providing necessary facilities to carry out this work.

LITERATURE CITED

1. Awah FM, Offor NN, Ndunaka AC, Okofor FU, Enyabine CO, 2012. Free radical scavenging activities and phenolic contents of the species *Thymus vulgaris* (Thyme), *Helichrysum italicum* (Curry leaf) and *Laurus nobilis* (Bay leaf) extracts. *J Pharmacy Res*, 5: 2994-2998.
2. Ames, B.N., Gold, L.S. and Willett, W.C. 1995. The causes and prevention of cancer. *Proc. Nat. Acad. Sci*, 92: 5258-5265.
3. Arya, V. and Yadav, J.P. 2011. Antioxidant properties of the methanol extracts of the leaves, seeds and stem of *Cassia occidentalis*. *Res. J. Med. Plant*, 5: 547-556.
4. Amarowicz, R., Pegg, R., Rahimi-Moghaddam, P., Barl, B. and Weil, J. 2004. Free-radical scavenging capacity antioxidant activity of selected plant species from the *Canadian prairies. Food Chem*, 84: 551-562.
5. Basniwal PK, Suthar M, Rathore GS, Gupta R, Kumar V, Pareek A, Jain D, 2009. *In vitro* antioxidant activity of hot aqueous extract of *Helieteres isora* Linn.fruits. *Nat Prod Radi*, 8: 483-487.
6. Bernatoniene J, Masteikova R, Davalgieno J, Pecinura R, Gauryliena R, Bernatoniene R, 2011. Tropical application of *Calendula officinalis* (L.); Formulation and evaluation of hydrophilic cream with antioxidant activity. *J Med Plant* Res, 5: 868-877.
7. Blois, M.S. 1958. Antioxidant determination by the use of a stable free radical. *Nat*, 181: 1199-1200.
8. Braugghler, J.M., Duncan, C.A. and Chase, L.R. 1986. The involvement of iron in lipid peroxidation. Important of ferrous to ferric iron in initiation. *J. Biol. Chem*, 261: 10282-10289.
9. Cerutti, P.A. 1991. Oxidant stress and carcinogenesis. *Euro. J. Clin. Invest*, 21: 1-5.
10. Cerutti, P.A. 1994. Oxy-radicals and cancer. *Lancet*, 344: 862-863.
11. Dolai N, Karmakar I, Suresh Kumar RB, Kar B, Bala A, Halder PK, 2012. Free radical scavenging activity of *Castanopsis indica* in mediating heptoprotective activity of carbon tetrachloride intoxicated rats. *Asian Pac J Trop Biomed*, 2: S243-S251.
12. Dheeraj, N.H., Patil, M.J.V. and Atul, M.W. 2010. *In vitro* antioxidant and *in vitro* antiinflammatory activity of *Cassia sophera* Linn. *Int. J. Pharm. Pharmaceu. Sci*, 2: 113-121.
13. Deighton, N., Brennan, R., Finn, C. and Davies, H.V. 2000. Antioxidant properties of domesticated and wild *Rubus* species. *J. Food Agric. Sci*, 80: 1307-1313.
14. Eom, S.H., Cheng, W.J., Hyoung, J.P., Kim, E.H., Chung, M.I., Kim, M.J., Yu, C. and Cho, D.H. 2007.

Far infra red ray irradiation stimulates antioxidant activity in *Vitis flexuosa* Thunb. Berries. *Kor. J. Med. Crop Sci*, 15: 319-323.
15. Ebadi, M. 2002. *Pharmacodynamic basis of Herbal Medicines* (CRC Press, Boca Raton), 86.
16. Fejes, S., Bla zovics, A., Lugasi, A., Lemberkovics, E., Petri, G. and Kery, A. 2000. *in vitro* antioxidant activity of *Anthriscus cerefolium* L. (Hoffm.) extracts. *J. Ethnopharmacol*, 69: 259-265.
17. Frankel, E.N., Waterhouse, A.L. and Teissedre, P.L. 1995. Principal phenolic phytochemicals in selected California wines and their antioxidant activity inhibiting oxidation of human low-density lipoprotein. *J. Agric. Food Chem*, 43: 890-894.
18. Gyamfi, M.A., Yonamine, M. and Aniya, Y. 1999. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. *General Pharmacol*, 32: 661-667.
19. Gupta, M., Mazumder, U.K., Gomathi, P. and Kumar, R.S. 2004 a. Antioxidant and free radical scavenging activities of *Ervatamia coronaria* Stapf. leaves. *Iranian. J. Pharm. Res*, 2: 119-126.
20. Harini R, Sindhu S, Sagadevan E, Arumugam P, 2012. Characterization of *in vitro* antioxidant potential of *Azadirachta indica* and *Abutilon indicum* by different assay methods. *J. Pharmacy Res*, 5: 3227-3231.
21. Hemmani, T. and Parihar, M.S. 1998. Reactive oxygen species and oxidative damage. *Indian. J. physiol. Pharmacol*, 42: 440-452.
22. Hinneburg, I., Dorman, D.H.J. and Hiltunen, R. 2006. Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem*, 97: 122–129.
23. Heinonen, M., Lehtonen, P.J. and Hopla, A. 1998. Antioxidant activity of berry and fruit wines and liquor. *J. Agric. Food Chem*, 48: 25-31.
24. Ilavarasan, R., Mallika, M. and Venkataraman, S. 2005. Antiinflammatory and antioxidant activities of *Cassia fistula* Linn bark extracts. *Afr. J. Trad. CAM*, 2: 70-85.
25. Jenecius AA, Uthayakumari F, Mohan VR, 2012. *In vitro* antioxidant of *Sauropus bacciformis* Blume (Euphorbiaceae) *Int Res J. Phar*, 3: 256-259.
26. Kanchanapoom T, Kamel MS, Kasai R, Picheansoonthon C, Hiraga Y, Yamasaki K, 2001. Benzoxazinoid glycosides from *Acanthus ilicifolius*. *Phytochem*, 58: 637-640.
27. Kumaran, A. and Karunakaran, J.R. 2007. *In-vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT Food SciTechnol*, 4: 344-352.
28. Kalpanadevi, V. and Mohan, V.R. 2012. *In vitro* antioxidant studies of *Begonia malabarica* Lam. and *Begonia flocifera* Bedd. *Asian Pac. J. Trop. Biomed*, 2: S386-S391.
29. Kahkonen, M.P., Hopia, A.I., Vuroela, H.J, Rauha, J.P., Pihlaja, K. and Kujala, T.S. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem*, 47: 3954-3962.
30. Kessler, M., Ubeaud, G. and Jung, L. 2003. Anti- and Pro-oxidant activity of rutin and quercetin derivatives. *J. Pharm. Pharmacol*, 55: 131-142.
31. Liu, F. and Ng, T.B. 2000. Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sci*, 66: 725-735.
32. Molan AL, Faraj AM, Mahdy A, 2012. Antioxidant activity and phenolic content of some medicinal plants traditionally used in Northern Iraq. *J Phytopharmacol*, 2: 224-233.
33. McDonald, S., Prenzler, P. D., Antolovich, M. and Robards, K. 2001. Phenolic content and antioxidant activity of olive extracts. *Food Chem*, 73: 73-84.
34. Marja, P.K., Anu, I.H., Heikki, J.V., Jussi-Pekka, R., Kalevi, P., Tytti, S.K. and Marina, H. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agri. Food. Chem*, 47: 3954-3962.
35. Mishra, S.S., Patel, K.K., Raghuwanshi, N., Path, A., Panda, P.P., Girhepunji, K. and Niranjan, C.H.P. 2011. Screening of ten Indian medicinal plant extracts for antioxidant activity. *Annals Biol. Res*, 2: 162-170.
36. Marxen, K., Vanselow, K.H., Lippemeier, S., Hintze, R., Ruser, A. and Hansen, U.P. 2007. Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. *Sensors*, 7: 2080–2095.
37. Murugan, M. and Mohan, V.R. 2012. *In vitro* antioxidant studies of *Dioscorea esculanta* (Lour) Brukill. *Asian Pac. J. Trop. Biomed*, 3: S1620-S1624.
38. Nishanthini, A. and Mohan, V.R. 2012. Antioxidant activities of *Xanthosoma sagittifolium* Schott using various *in vitro* assay models. *Asian Pac. J. Trop. Biomed*, 2: S1701-S1706.
39. Pietta, P.G. 2000. Flavonoids as antioxidant. *J. Nat. Pro*, 63: 1035-1042.
40. Preethi, K.C., Kuttan, G. and Kuttan, R. 2006. Antioxidant potential of an extract of *Calendula officianalis* flowers *in vitro* and *in vivo*. *Pharm. Biol*, 44: 691-697.
41. Ravikumar S, Gnanadesigan M, Suganthi P, Ramalakshmi, 2010. Antibacterial potential of chosen mangrove plants against isolated urinary tract infectious bacterial pathogens. *Int J. Med Sci*, 2: 94-99.
42. Ravikumar S, Gnanadesigan M, 2011. Hepatoprotective and antioxidant properties of marine halophyte *Luminetzera racemosa* bark extract in CCl4 hepatotoxicity. *Asian Pac J. Trop. Biomed*, 1: 348-352.
43. Re, R., Pellegrimi, N., Proleggenke, A., Pannala, A., Yang, M. and Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free. Rad. Biol. Med*, 26: 1231-1237.

44. Sharmila Jose G, Radhamani PM, 2012. Identification and determination of antioxidant constitutions of bioluminescent mushroom. *Asian Pac J Trop Biomed*, S386-S391.
45. Subasree M, Mala P, Uma maheshwari M, Jeya kumara M, Maheshwari K, Sevanthi T, Manikandan T, 2010. Screening of the antibacterial properties of *Avicennia marina* from Pitchavaram Mangroves. *Int. J Curr Res*, 1: 16-19.
46. Shirwaikar, A., Kuppusamy, R. and Punitha, I.S.R. 2006. *In-vitro a*ntioxidant studies on the Benzyl Tetra Isoquinoline alkaloid Berberine. *Biol Pharm Bull*, 29: 1906-1910.
47. Sanchez-Moreno, C. 2002. Review: methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food. Sci. Tech. Int*, 8: 121-137.
48. Sainani, G.S., Manika, J.S. and Sainani, R.G. 1997. Oxidative stress: a key factor in pathogenesis of chronic disease. *Med Uptake*, 1: 1-4.
49. Sreejayam, M. and Rao, M.N.A. 1996. Free radical scavenging activity of *Cucuminoids*. *Arzneim-Forsch Drug Res*, 46: 169-171.
50. Singh, N. and Ranjini, P.S. 2004. Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem*, 85: 611-616.
51. Siddhuraju, P., Mohan, P. and Becker, K. 2002. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chem*, 79: 61-67.
52. Shahidi, F. and Wanasundara, P.K.J.P.D. 1992. Phenolics antioxidants. *Critical Rev. Food Sci. Nutri*, 32: 67-103.
53. Tapia, A., Rodriguez, J., Theoduloz, C., Lopez, S., Feresin, G.E. and Schmeda-Hirshmann, G. 2004. Free radical scavengers and antioxidants from *Baccharis grisebachii*. *J. Ethanopharmacol*, 95: 155-161.
54. Tresina, P.S., Mary Jelastin Kala, S, and Mohan, V.R. 2012a. HPTLC finger print analysis of phytocompounds and in vitro antioxidant activity of *Eugenia floccosa* Bedd. *Biosci. Discov*, 3: 296-311.
55. Tresina, P.S., Mary Jelastin Kala, S, and Mohan, V. R. 2012b. HPTLC finger print analysis of phytocompounds and *in vitro* antioxidant activity of *Eugenia singampattiana* Bedd. *J. Appl. Pharmaceu. Sci*, 2: 112-124.
56. Velioglu, Y.S., Mazza, G., Gao, L. and Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem*, 46: 4113-4117.
57. Vinson, J.A., Hao, Y. and Zubic, S.K. 1998. Food antioxidant quantity and quality in foods: Vegetables. *J. Agric. Food Chem*, 46: 3630-3634.
58. Wang, T., Jonsdottir, R. and Olafsdottir, G. 2009. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic sea weeds. *Food Chem*, 116: 240-248.
59. Xu N, Fan X, Yan X, Li X, Niu R, Tseng CK, 2004. Antibacterial bromophenols from the marine red algae *Rhodomela conferoides*. *Phytochem*, 62: 1221-1224.
60. Yokozawa, T., Chen, C.P., Dong, E., Tanaka, T., Nonaka, G.I. and Nishioka, I. 1998. Study on the Inhibitory Effect of Tannins and Flavonoids against the 1,1-Diphenyl-2-picrylhydrazyl Radical. *Biochem. Pharmacol*, 56: 213-222.
61. Yen, G., Duh, P. and Tsai, C. 1993. Relationship between antioxidant activity and maturity of peanut hulls. *J. Agric. Food Chem*, 41: 67-70.
62. Yen, G.C. and Chen, H.Y. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem*, 43: 27-32.
63. Yildirim, A., Mavi, A., Oktay, M., Kara, A.A., Algur, O.F. and Bilaloglu, V. 2000. Comparison of antioxidant and antimicrobial activities of *Tilia* (*Tilia argentea* Desf Ex DC), Sage (*Salvia triloba* L.), and bark Tea (*Camellia sinensis*) extracts. *J. Agirc. Food Chem*, 48: 5030-5034.