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Antioxidant Potential of Aerial Part of *Senna italica* Sub Species *micrantha* Mill.

R.Shunmuga jothi [1], V.Bharathy [2] & F.Uthayakumari[3]

[1&3] *Research Centre for Plant Sciences, Department of Botany, St.Mary's college (Autonomous) Thoothukudi,Tamil Nadu.*
[2] *Department of Botany, Fatima college(Autonomous) Madurai, Tamil Nadu*

Abstract

The aim of the study is to screen various solvent fraction of aerial parts of *Senna italica* ssp *micrantha* to display *in vitro* antioxidant activity using DPPH assay, hydroxyl assay, ABTS assay, superoxide assay, reducing power assay. Petroleum ether fraction show high potential for DPPH assay and methanol fractions show high antioxidant activity for other assays. The radical scavenging abilities are found to be dose dependant. IC_{50} values of methanol extracts of the species studied for DPPH, hydroxyl, ABTS, superoxide radical scavenging activity compared with standard ascorbic acid and are found to be 38.18µg/ml, 31.74µg/ml, 43.18µg/ml and 43.64µg/ml respectively. The results indicate that the plant sample studied have antioxidant property and these activities observed can be the synergistic effects of phytocompound present in the plant.

Keywords: Antioxidant activity, *Senna italica* ssp *micrantha.*

INTRODUCTION

Reactive oxygen species (ROS) are produced in all aerobic cells as by-products of oxygen metabolism. When ROS generation overwhelms the cellular antioxidant capacity it results in oxidative stress, which ultimately leads to cell death or transformation [1,2]. Antioxidant compounds can decrease oxidative stress and minimise the incidences of diseases like cancer, heart diseases and gastric problems etc. The mechanism of action of these antioxidant compounds include suppression of reactive oxygen species formation, inhibition of the enzymes or by chelating of trace elements involved in free radical production , scavenging of reactive species and up-regulating or protecting antioxidant defence. Recent studies have shown that a number of plant products including polyphenols, terpenes and various phytoconstituents exerted an antioxidant action [3].There is currently immense interest in plant antioxidants and their role in human health and nutrition. Most of the food plants around the globe have been screened for their antioxidant proper and the knowledge about the antioxidant ability of the medicinal plants are only fragmentary.

Senna species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. They are well known in folk medicine for their laxative and purgative uses [4]. Besides ,they have been found to exhibit anti –inflammatory ,antioxidant [5], hypoglycemic [6] , antiplasmodial, larvicidial [7], antimutagenic [8,9] ,and anticancer activities [10]. The present study is designed to evaluate the antioxidant and scavenging potential of aerial parts of *Senna italica* ssp *micrantha.*

MATERIALS AND METHODS

Collection of plant sample

The plant material is collected from areas of Thoothukudi, Tamil Nadu. The plant is identified and authenticated by Botanical Survey of India, Southern circle Coimbatore as *Senna italica* ssp *micrantha* Mill. (Caesalpiniaceae) voucher specimen(SMCH-33527) is preserved in Department of Botany, St.Mary's College (Autonomous), Thoothukudi.

Preparation of powder and extract

The aerial parts of the plants are shade dried and pulverized to powder in a mechanical grinder . The powder(100g) is extracted successively with methanol, ethanol, petroleum ether, benzene and ethyl acetate, each 250ml in a soxhlet apparatus [11]for 48 hrs. All the extracts are filtered through Whatman no: 41 filter paper and are concentrated in a rotary evaporator. The concentrated extracts are used for *in vitro* antioxidant activity.

DPPH Radical Scavenging [12]

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H. The free radical scavenging activity of all the extracts are evaluated by 1, 1-diphenyl-2- picryl-hydrazyl (DPPH). An 0.1 mm solution of DPPH in methanol is prepared, and 1 ml of this solution is added to 3 ml of all extracts in methanol at different concentrations (125, 250, 500 and 1000 µg/ml). The mixtures are shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbances is measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10 UV: Thermoelectron Corporation). Ascorbic acid is used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging DPPH radical is calculated by using the following formula:

DPPH scavenging effect (% inhibition) = $(A_0 – A_1) \times 100/A_0$

Here, A_0 is the absorbance of the control reaction and A1 is the absorbance in presence of all of the extract samples and

reference. All the tests are performed intriplicates and the results are averaged.

Hydroxyl radical scavenging activity [13]

The scavenging capacity for hydroxyl radical is measured according to the standard method. Stock solutions of EDTA (Ethylene diamine tetraacetic acid) (1mM), Fecl3 (10mM), Ascorbic Acid (1mM), H2O2 (10mM) and Deoxyribose (10 mM), are prepared in distilled deionized water. The assay is performed by adding 0.1ml EDTA , 0.01ml of FeCl3**,** .1ml H2O2, 0.36mL of deoxyribose, 1.0 ml of the extract of different concentration (50,100,200,400 and 800µg/ml) dissolved in distilled water, 0.33ml of phosphate buffer (50mM , pH 7.4), 0.1ml of ascorbic acid in sequence . The mixture is then incubated at 37oc for 1 hour. 1.0ml portion of the incubated mixture is mixed with 1.0 mL of 10%TCA and 1.0ml of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using the following equation.

Hydroxyl radical scavenging activity = {(A_0 –A_1)/A_0)*100}

Where, A_0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference. All the tests are performed in triplicates and the results are averaged.

Superoxide radical scavenging activity [14]

The superoxide anion radicals are generated in 3.0 ml of Tris – HCL buffer (16 mM, PH 8.0), containing 0.5 ml of Nitro Blue Tetrazolium (NBT) (0.3mM), 0.5 ml NADH (0.936mM) solution, 1.0 ml extract of different concentration (50,100,200,400 & 800µg/ml), and 0.5 ml Tris – HCl buffer (16mM, pH 8.0). The reaction is started by adding 0.5 ml PMS (Phenazine methosulphate) solution (0.12mM) to the mixture, incubated at 25oC for 5 min and the absorbance is measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition is calculated by using the following equation.

Superoxide radical scavenging activity= {(A_0 –A_1)/A_0)*100}

Where, A_0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference. The entire test is performed in triplicates and the results are averaged.

Antioxidant Activity by Radical Cation (ABTS +) [15]

ABTS assay is based on the slightly modified method of Huang *et al.,* 2011.

ABTS radical cation (ABTS+) is produced by reacting 7mM ABTS (2,2'-azino-bis(3- ethylbenzothiazoline-6-sulphonic acid) solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use.The ABTS + Solution are diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of 100µl of sample or trolox standard to 3.9 ml of diluted ABTS+ solution, absorbance is measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results are expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = {(A_0 –A_1)/A_0)*100}

Where, A_0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference. All the tests are performed in triplicates and the results are averaged.

Reducing Power[16]

The reducing power of the extract is determined by standard methods. 1.0 ml of solution containing 50,100,200,400 and 800µg/ml of extract is mixed with sodium phosphate buffer (5.0 ml, 0.2 M, pH6.6) and potassium ferricyanide (5.0 ml, 1.0%). The mixture is incubated at 50oC for 20 minutes. Then 5ml of 10% trichloroacetic acid is added and centrifuged at 980 x g (10 minutes at 5^{o}C) in a refrigerator centrifuge. The upper layer of the solution (5.0 ml) is diluted with 5.0 ml of distilled water and ferric chloride and absorbance read at 700 nm. The experiment is performed thrice and results are averaged.

RESULT AND DISCUSSION

In the present study the antioxidant activity of methanol, ethanol, petroleum ether, benzene and ethyl acetate fraction are assessed using DPPH assay, hydroxyl assay, ABTS assay, superoxide dismutase assay and reducing power. IC_{50} values are shown in the table 6.

There are many different experimental methods to assess free radical scavenging activity. One such method for evaluating total free radical scavenging activity is DPPH radical scavenging assay. Methanol, ethanol, petroleum ether, benzene and ethyl acetate the extracts of *Senna italica* ssp *micrantha* the scavenging effect increases with concentration of standard and sample. Among the solvent tested , methanol extract (800µg/ml) exhibited highest DPPH radical scavenging activity (118.16%) than the standard ascorbic acid whose scavenging effect is 106.26% . In DPPH assay IC_{50} 39.84µg/ml values of petroleum ether solvent show highest value than that of other solvents. DPPH react with suitable reducing agents, the electrons become paired off and the solution loss colour stoichiometrically depending on the number of electrons taken up. A large decrease in absorbtion of reaction mixture indicate significant free radical scavenging activity[17].(table 1 & fig 1)

Hydroxyl radical is one of the potent reactive oxygen species in the biological system. Hydroxyl radical is regarded as a detrimental species for pathophysiological processes and capable of damaging every biomolecules and it leads to carcinogenesis, mutagenesis and cytotoxicity [18]. Hydroxyl radical scavenging activity is directly proportional to its antioxidant activity which is depicted by the intensity of red colour [19].The methanolic extract of the selected plant scavenge hydroxyl radical more activity 113.15% than other extracts(table 2 & fig 2) .

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6- sulfonic acid) assay is an excellent tool for measuring antioxidant activity of phytochemical products . The reduction capability of ABTS radical is determined by the decrease in its absorbance at 734nm which is induced by antioxidant . The methanolic extracts of the selected taxon show potent

antioxidant activity. The antioxidant activity is dose dependent and more in methanolic extract (table 3& fig 3).
Superoxide radical is very harmful to cellular compound and it is considered as a major biological source of reactive oxygen species[20]. All solvent extracts exhibit superoxide scavenging activity but the methanolic extract has effective capacity of scavenging for superoxide radical and it be correlated with flavanoid content and suggesting its antioxidant potential as observed by Saeed *et al*[21].(table 4 & fig 4)
Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes , so that they can act as primary and secondary antioxidant . Table 5 & fig 5 showed the reducing ability of different extracts of *S.italica* ssp *micrantha* compared to ascorbic acid. The results clearly indicate that the reducing power of the selected plant increase in dose dependent manner . Among the solvent tested extract methanol extract exhibited higher reducing activity (0.512%)

Table 1: Effect of different solvent extract of *Senna italica* ssp *micrantha* on DPPH assay

concentration	DPPH radical scavenging activity %					
	Methanol	Ethanol	Petroleum ether	Benzene	Ethyl acetate	Standard (Ascorbic acid
50	32.46±0.24	27.16±0.24	29.31±0.67	19.36±0.34	26.11±0.39	29.13±0.36
100	53.91±0.18	48.74±0.37	46.36±0.84	28.11±0.27	42.84±0.12	46.16±0.27
200	79.16±0.39	65.12±0.16	66.39±0.57	40.65±0.18	65.14±0.26	73.09±0.13
400	94.13±0.45	87.16±0.39	82.13±0.45	61.22±0.24	84.13±0.11	89.11±0.25
800	118.16±0.35	104.33±0.18	108.16±0.13	93.18±0.18	108.16±0.32	106.26±0.13

Table 2: Effect of different solvent extract of *Senna italica* ssp *micrantha* on hydroxyl assay

concentration	Hydroxyl radical scavenging activity %					
	Methanol	Ethanol	Petroleum ether	Benzene	Ethyl acetate	Standard (Ascorbic acid
50	31.43±0.15	26.81±0.34	21.65±0.62	18.34±0.16	22.31±0.67	28.36±0.16
100	46.13±0.34	41.06±0.13	37.16±0.84	29.18±0.24	43.11±0.24	52.11±0.83
200	73.91±0.56	43.11±0.37	56.13±0.16	46.13±0.16	59.22±0.16	68.16±0.11
400	91.56±0.27	81.16±0.15	73.81±0.27	64.28±0.26	78.36±0.24	83.84±0.26
800	113.15±0.81	104.31±0.27	96.11±0.34	82.11±0.34	93.46±0.18	101.36±0.13

Table 3: Effect of different solvent extract of *Senna italica* ssp *micrantha* on ABTS assay

concentration	ABTS radical scavenging activity %					
	Methanol	Ethanol	Petroleum ether	Benzene	Ethyl acetate	Standard (Ascorbic acid
50	30.92±0.16	28.36±0.24	23.41±0.22	16.24±0.18	22.96±0.26	28.91±0.37
100	49.84±0.26	43.18±0.16	36.84±0.27	29.92±0.27	40.18±0.12	43.76±0.13
200	76.92±0.18	74.19±0.26	58.33±0.19	46.13±0.16	73.16±0.24	70.13±0.27
400	96.12±0.24	91.16±0.39	80.24±0.24	68.93±0.21	91.42±0.16	89.22±0.63
800	124.13±0.83	112.24±0.21	98.42±0.19	90.22±0.46	113.84±0.24	104.13±0.84

Table 4: Effect of different solvent extract of *Senna italica* ssp *micrantha* on Superoxide dimutase assay

concentration	Superoxide radical scavenging activity %					
	Methanol	Ethanol	Petroleum ether	Benzene	Ethyl acetate	Standard (Ascorbic acid
50	29.31±0.42	24.92±0.26	26.67±0.46	19.84±0.26	21.92±0.63	28.39±0.36
100	48.96±0.26	38.84±0.18	42.81±0.24	30.26±0.16	43.67±0.84	43.16±0.24
200	73.16±0.39	55.46±0.21	63.86±0.12	49.84±0.27	78.24±0.16	74.92±0.13
400	96.05±0.43	73.46±0.92	84.27±0.92	73.80±0.16	96.27±0.24	89.36±0.24
800	126.84±0.27	94.22±0.24	106.26±0.26	98.26±0.19	112.92±0.27	106.37±0.18

Table 5: Effect of different solvent extract of *Senna italica* ssp *micrantha* on reducing power

concentration	Reducing power OD					
	Methanol	Ethanol	Petroleum ether	Benzene	Ethyl acetate	Standard (Ascorbic acid
50	0.334±0.076	0.318±0.026	0.193±0.026	0.242±0.031	0.286±0.024	0.288±0.031
100	0.376±0.031	0.367±0.031	0.248±0.013	0.291±0.042	0.318±0.016	0.334±0.024
200	0.413±0.028	0.396±0.018	0.290±0.036	0.340±0.054	0.367±0.026	0.368±0.011
400	0.456±0.037	0.442±0.021	0.339±0.073	0.393±0.096	0.408±0.031	0.402±0.024
800	0.512±0.041	0.496±0.039	0.391±0.024	0.434±0.027	0.468±0.076	0.458±0.018

Table 6: IC50 values for different solvent extracts of aerial parts of *Senna italica* ssp *micrantha*

IC50 µg/ml				
Different solvent extract	**DPPH assay**	**Hydroxyl assay**	**ABTS assay**	**Superoxide dimutase assay**
Methanol	38.18	31.74	43.18	43.64
Ethanol	35.96	27.03	37.11	31.49
Petroleum ether	39.84	23.25	29.28	38.22
Benzene	34.54	24.92	27.19	34.62
Ethyl acetate	38.56	26.37	36.84	40.88
Standard (Ascorbic acid)	36.22	28.28	32.13	36.22

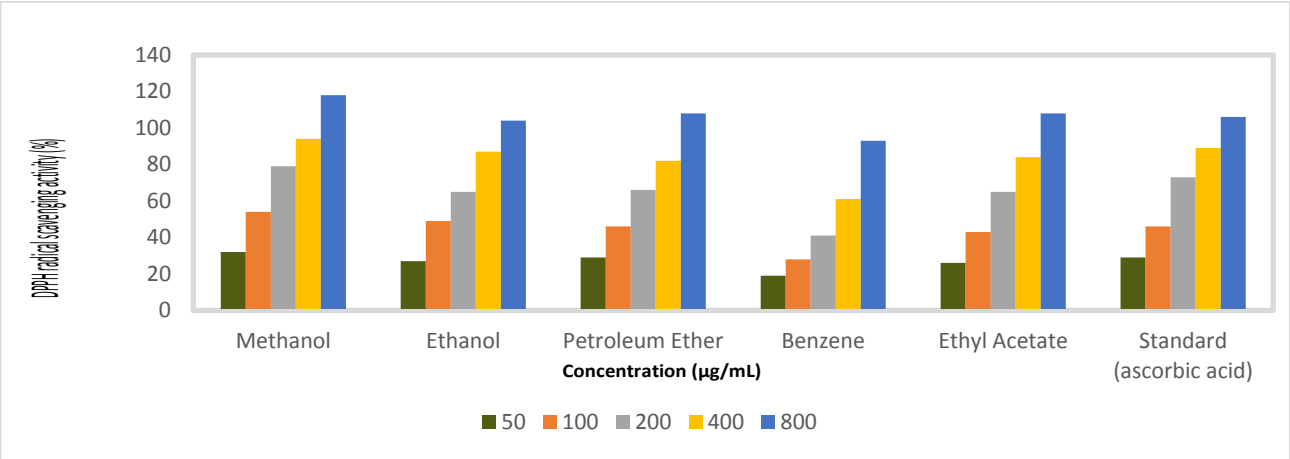

Fig 1:DPPH radical scavenging activity

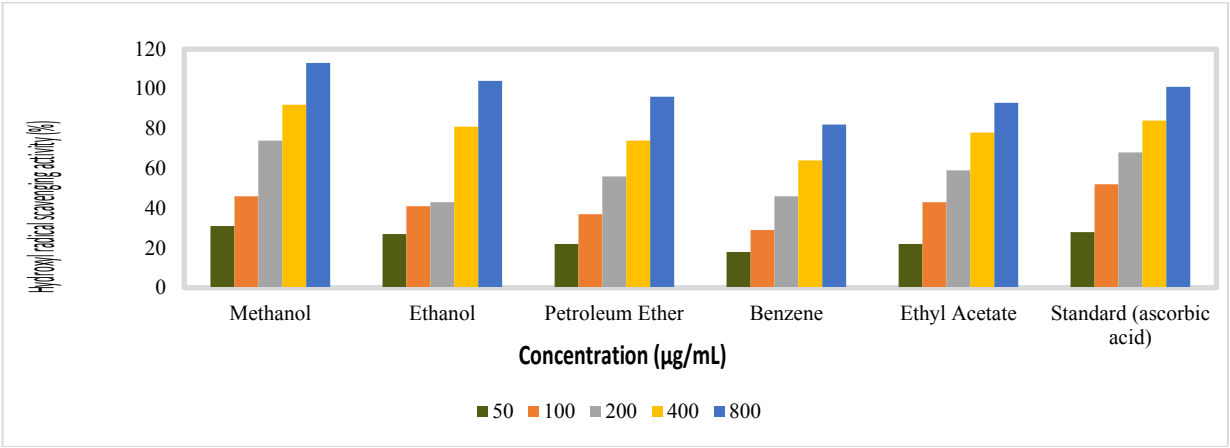

Fig 2:Hydroxyl radical scavenging activity

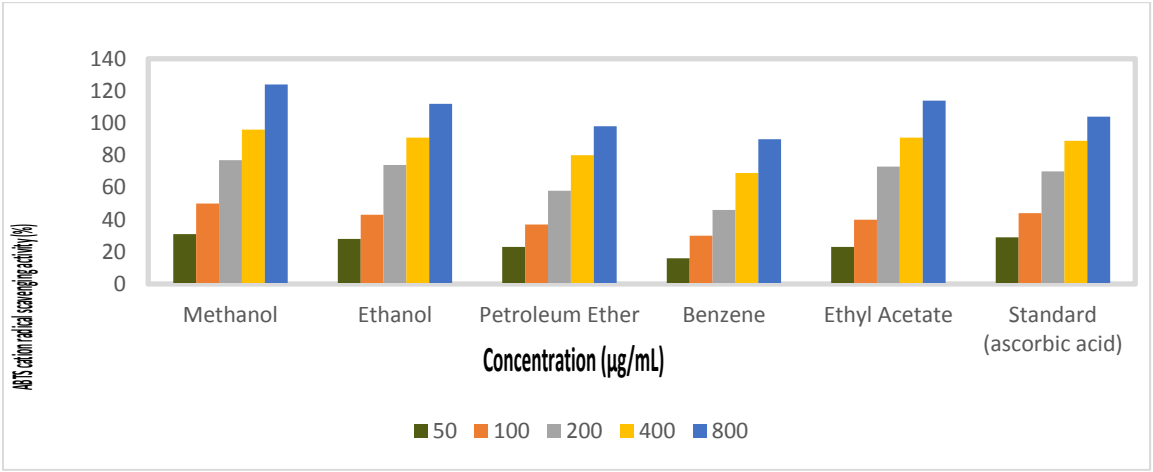

Fig 3:ABTS radical scavenging activity

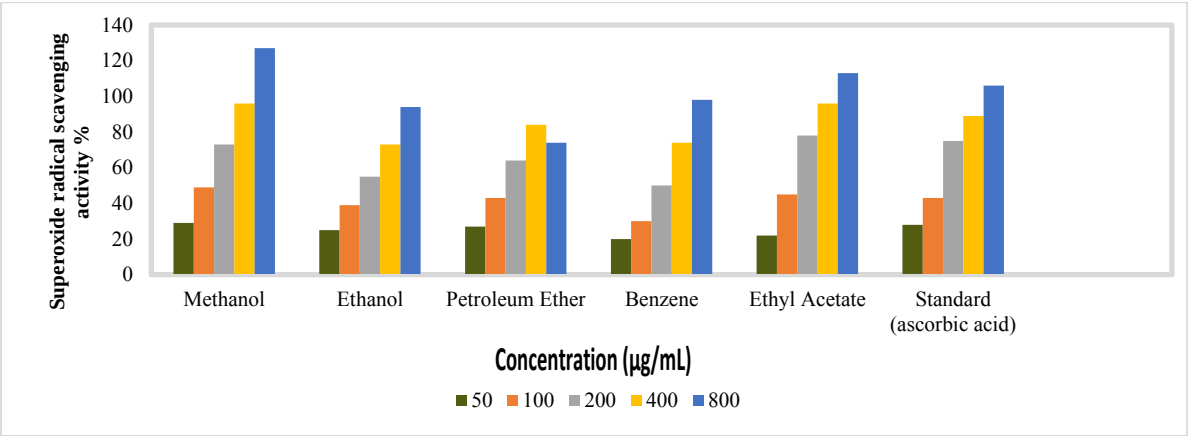

Fig 4:Superoxide dismutase radical scavenging activity

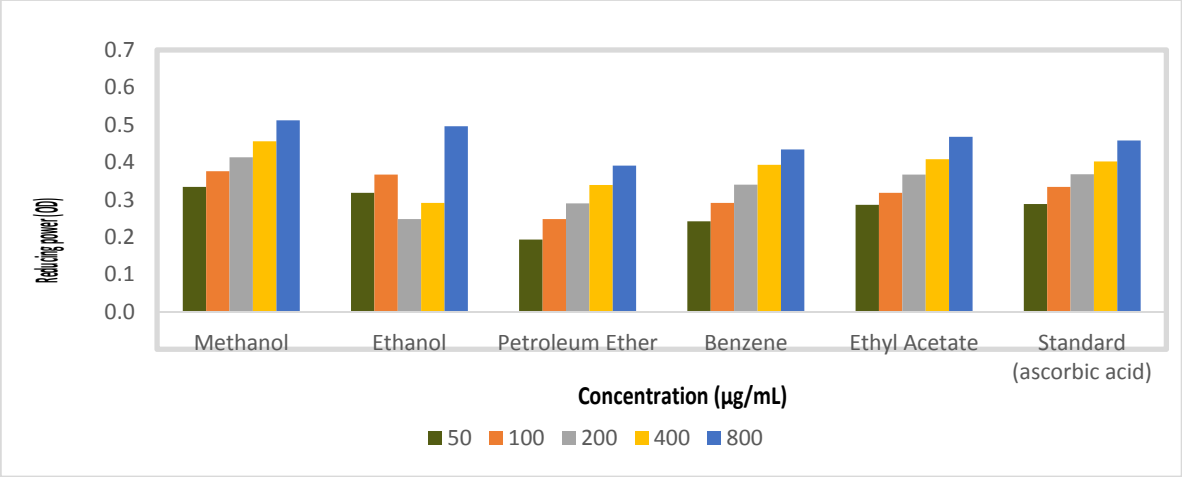

Fig 5: Reducing power

CONCLUSION

The results indicate that the selected taxon is more potential as an antioxidant . The antioxidant activity could be attributed to phenolic compounds especially flavonoids which possess antioxidant action [22] or it can be the synergistic effect of phytocompounds that present in the plant.

REFERENCES

[1] Sies H, *Oxidative Stress: Oxidants and Antioxidants*, Academic Press, London, 1991, 276-280.

[2] Goldfarb, A.H., *Medicine and Science in Sports and Exercise.*1993, *25*, 232-236 .

[3] Manickam ,M., Ramanathan, M., Jahromi, F.M.A., *Journal of Natural Product*, 2007, *60*, 609-610.

[4] Hennebelle, T., Weniger, B., Joseph, H., Sahpaz, S., Bailleul ,F., *Senna alata*. *Fitoterapia.* 2009,*80*, 385-393.

[5] Yen, G.C., Chuang, D.Y. , *J. Agric. Food Chem*., 2000, *48*, 2760-2765.

[6] Jalalpure, S.S, Patil, M.B., Pai, A., Shah, B.N., Salahuddin, M.D., *Nig. J. Nat. Prod. Med*., 2004, *8*, 22-23.

[7] Yang, Y., Lim ,M., Lee, H., *J. Agric. Food Chem*., 2003,*51*, 7629-7631.

[8] Silva,C.R., Monteiro, M.R., Rocha, H.M., Ribeiro, A.F., Caldeira-de-Araujo, A., Leitao, A.C., Bezerra, R.J.A.C., Padula, M., *Toxicol.*,2008 22: 212-218.

[9] Yadav, J.P., Arya, V., Yadav, S., Panghal, M., Kumar, S., Dhankhar, S., *Fitoterapia*, 2010, *81*, 223-230.

[10] Prasanna, R., Harish, C.C., Pichai, R., Sakthisekaran, D., Gunasekaran, P., *Cell Biol. Int*, 2009, *33*, 127-134.

[11] Handa, S.S., Khanuja, S.P.S., Dev, G.L., Rakesh ,D., Extraction Technologies for Medicinal and Aromatic Plants. *United Nations Industrial Development Organisation and international centre for Science and High Technology*,Trieste,2008.

[12] Shen, Q., Zhang, B., Xu, R., Wang, Y., Ding, X., Li, P., *Bifodobacterium animalis* , Anaerobe. 2010; *16*, 380-386.

[13] Halliwell, B., Gutteridge, J.M.C., Aruoma, O.I., The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. *Ana Biochem*., 1987, *65*, 215-219.

[14] Srinivasan, R., Chandrasekar, M.J.N., Nanjan, M.J., Suresh, B., *J Ethnopharm*, 2007,*113*, 284-291.

[15] Huang, M.H., Huang, S.S., Wang, B.S., Sheu, M.J., Hou, W.C., *J Ethnopharm*, 2011, *113*, 743-750.

[16] Kumar, R.S., Hemalatha. S., *J Chem Pharm Res*, 2011, *3*, 259-267.

[17] Krishnaiah , D., Sarbatly , R., Nithyanandan, R,R., *Food and Bioproducts Processing*, 2011, *89*, 217-233.

[18] Babu, B.H., Shylesh, B.S., Padicala, J., *Filoterapia.* 2001, *72*, 272-277.

[19] Gulcin, I., Alici, H.A., Cesur, M., *Chemical and Pharmaceutical Bulletin.* 2005,*53*,281- 285.

[20] Alves, C.Q., David , J.M., David ,J.P ., Bahia, M.V., Agular , R.M., *Ouimica niva.* 2010, *33*, 2202-2210.

[21] Saeed, N., Khan, M.R., Shabbir, M., 2012. *Complementary and adternative Medicine.* 2012, *12*, 221.

[22] Anandakumar ,A.M., Paulsamy, S., Sathiskumar, P., Sethilkumar ,P., *J.Appl.Nat.Sci.* 2009, *1*, 41-43.