Original Research Article

A Study on the Antioxidant Properties of Some Selected Medicinal Plants from Kandhamal District of Odisha, India.

ABSTRACT

Antioxidant properties of medicinal plants of Kandhamal district of Odisha have not reported, while these plants are used to treat different ailments such as malaria, rheumatoid, cold and cough, piles, diarrhoea and tooth ache etc. The present work was aimed to evaluate the total phenolic and flavonoid contents as well as in vitro antioxidant properties of methanolic extract of medicinal plants. The total phenolic and flavonoid contents were determined by Folin- Ciocalteu and, aluminium chloride method with standard gallic acid and rutin respectively. Antioxidant activity was determined by 1, 1- diphenyl- 2- picrylhydrazyl (DPPH), hydroxyl radical, superoxide radical, and hydrogen peroxide assay. The total phenolic content of medicinal plants varied from 44.53 to 297.07 mg/g while the flavonoid content was varied from 14.03 to 60.49 mg/g. In conclusion, these plants are rich sources of phenolic compounds and antioxidants and they could be used as a natural antioxidant.

Medicinal plants, Phenolics, antioxidant properties, Kandhamal district.

1. INTRODUCTION

Plants and plants derived products are used by men for their basic requirements. The use of traditional medicine is wide spread and plants are still a large source of natural antioxidants that might serve as leads for the development of novel drugs [1]. In fact, medicinal plants are considered to be the main sources of several phytochemical compounds like alkaloids, tannins, phenols, saponin, coumarins, steroids and flavonoids curing diversified chronic diseases [2]. The most obvious role of phytochemical compounds is protection from free radicals or reactive oxygen species (ROS) that is produced continuously in human body [3]. Free radicals or ROS were generated during metabolism resulting in oxidative stress. Oxidative stress occurs when the balance between free radical and antioxidants is disrupted by excessive production of ROS, and/or inadequate antioxidant defenses [4]. Generally, there is a stability between the number of free radicals are produced in the body and the antioxidant defense systems, which scavenge these free radicals [5]. When the free radicals amount is

within the standard physiological level and preventing them from causing adverse effects in the body [2], but when this balance is shifted towards more free radicals, it leads to oxidative stress and causes various degenerative diseases such as cancer, aging, coronary heart diseases, and gastric ulcer [6, 7]. It is probably to reduce such diseases by either enhancing the body's natural antioxidant defenses or by supplementing with dietary antioxidants [8]. There are four most synthetic antioxidants are used such as butylated hydroxytoluene (BHT), propyl gallate (PG), butylated hydroxyanisole (BHA) and *tert*-butylhydroxyquinone (TBHQ) [9]. But now-a-days these chemicals are responsible for liver damage and acting as carcinogenesis in laboratory animals [10]. Therefore, the development and utilization of more effective antioxidants of natural origin are desirable [11]. These antioxidants occur in various plants, which are having high or less antioxidant capacity those may trace out *in vitro* way analysis. Therefore, the present work was aimed to evaluate the total phenolic and flavonoid content as well as *in vitro* antioxidant properties of methanolic extract of medicinal plants.

2. MATERIALS AND METHODS

2.1 Collection of plant materials

Nyctanthes arbor-tristis (NA), Tinospora cordifolia (TC), Phyllanthes niruri (PN), Andrographis paniculata (AP), Lantana camara (LC), Mimosa pudica (MP), Justicia adhatoda (JA), Cuscuta reflexa (CR), Cyperus rotendus (CyR), Piper nigrum(PiN), Ocimum sanctum (OS), Cantharanthus roseus (CaR) and Clitoria ternatea (CT) are the plant samples were used in the present investigation (Table 1). The plant materials were collected in the month of December 2016 to January 2017 from Kandhamal district (coordinates 20.13^0 N and 84.01^0 E) of Odisha, India. The plants were identified by the specialists and deposited in the Herbarium house; Department of Botany, Berhampur University, Odisha, India.

2.2 Extraction of plant materials

The selected plant parts were removed from the plants and then washed under running tap water to remove dust. The plant samples were then oven dried at 60^0C for few days and was crushed into powders in a mechanic grinder. The powdered materials were then extracted using solvent methanol (300 mL) through the Soxhlet apparatus. After extracting all coloring materials, the filtrate was concentrated by evaporating in a water bath under normal pressure. The dried extracts were weighed to determine the percent of yield using the formula [12].

$$\text{Extract yield } \% = W2 - W1/W0 \times 100$$

Where, W2= the weight of the extract and the container, W1= the weight of the container alone and W0= the weight of the initial dried sample.

The dried extracts were stored at 4^0C for further investigation of potential *in vitro* free radical scavenging activity.

2.3 Estimation of Total Phenolic and flavonoid contents

The total phenolic content (TPC) was analyzed by the Folin-Ciocalteu colorimetric method using gallic acid as a standard and expressed as mg/g gallic acid equivalent [13]. Similarly, the total flavonoid content (TFC) was analyzed using rutin as a standard and this was expressed as mg/g rutin [14].

2.4 Antioxidant assay

2.4.1 DPPH (*1, 1-Diphenyl-2-picryl-hydrazyl*) radicals scavenging activity

The free radical scavenging capacities of the methanolic extracts were determined by using DPPH assay [15]. DPPH solution (0.004%, w/v) was prepared in methanol. Stock solution (1mg/mL) of methanolic extract of plant and standard ascorbic acid (0.05g/mL) were prepared using methanol. Various concentrations (10-500 µg/mL) of the plant extract and ascorbic acid were taken in test tube and 1mL freshly prepared DPPH solution were added, the test tubes were protected from light by covering with aluminium foil. The final volume in each test tube was made to 2mL with methanol and incubated in dark for 30 mins at room temperature. After incubation the absorbance was read at 517 nm using a spectrophotometer. Control sample was prepared containing the same volume of methanol and DPPH without any extract and reference ascorbic acid. Methanol was served as blank. The radical scavenging was calculated by the following formula;

% Inhibition = [(Abs of control – Abs of sample)/ Abs of control] x 100

2.4.2 Hydroxyl radical scavenging activity

The reaction mixture (3 mL) containing 1 mL $FeSO_4$ (1.5 mM), 0.7 mL hydrogen peroxide (6 mM), 10% of 0.3 mL sodium salicylate (20 mM) and varying concentrations of the extracts (10-500µg/mL) were taken. After incubation for 1hr at 37^0C, the absorbance of the hydroxylated salicylated complex was measured at 562 nm [16]. Ascorbic acid was used as the standard. The percentage scavenging effect was calculated as:

% scavenging activity = [1- (A1 – A2)/ A0] × 100, where A0 was the absorbance of the control (without extract), A1 was the absorbance in the presence of the extract with sodium salicylate, and A2 was the absorbance without sodium salicylate.

2.4.3 Superoxide anion radical scavenging activity

This assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS) under aerobic condition [17]. Tris HCl buffer (3mL, 16 mM, pH 8.0) containing 1 mL NBT (50 µM) solution, 1 mL NADH (78 µM) solution and a sample solution of extract (10-

500µg/mL) in distilled water mixed. The reaction was started when 1 mL of PMS solution (10 µM) was added to the mixture. The reaction mixture was incubated at 25^0C for 5 min, and the absorbance was read at 560 nm against the corresponding blank samples. Ascorbic acid was used as a standard. The decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity.

2.4.4 Hydrogen peroxide radical scavenging activity

The capability of the extract to scavenge hydrogen peroxide (H_2O_2) was estimated according to the method of Nabavi *et al.* [18]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer, pH 7.4. The concentration of hydrogen peroxide was determined by absorption at 230 nm using a UV- visible spectrophotometer. The extracts (10-500µg/mL) in distilled water were added to a hydrogen peroxide solution at 230 nm was determined after 10 mins against the blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as a standard.

2.5 Statistical Analysis

All experimental measurements were carried out in triplicate and are expressed as average of three analysis ± standard deviation (SD).

3. RESULTS

3.1 Percentage of Yield

Table 2 showed the percentage of yield of methanolic extracts of different plant parts. In the methanolic extract, *C. ternatea* gave the highest yield (38%) while *C. roseus* gave the least yield (24.4%).

3.2 Total Phenolic and Flavonoid Contents

The total phenolic contents (mg/g) in methanolic extracts were examined from the regression equation expressed in gallic acid equivalents (GAE). The total phenolic content of methanolic extracts of medicinal plants as shown in Table 2. The highest amount was found in the extract of *T. cordifolia* (294.07 ± 0.37 mg/g) and the minimum amount was found in *M. pudica* (44.53 ± 0.05 mg/g). The concentration of total flavonoid content in different plant extracts were determined by using the spectrophotometric method with aluminium chloride. The total flavonoid contents were expressed in terms of rutin equivalent (regression equation of calibration curve: y= 0.005x + 0.005, R2 = 0.998), mg of RU/g of extracts. The total flavonoid contents ranged from 5.63 ± 0.03 to 85.43 ± 0.25 mg/g (Table 2).

3.3 Antioxidant assay

3.3.1 DPPH free radical scavenging activity

This assay is based on the scavenging of the DPPH radical from the antioxidants, which produces a decrease in absorbance at 517 nm. The highest amount of antioxidant activity of

the methanolic extract was found in *C. reflexa* (91.04%) and the minimum antioxidant activity was found in *A. paniculata* (41.71%) at the concentration of 500µg/mL. The result of DPPH inhibition by different plant extracts are summarized in Fig. 1.

3.3.2 Hydroxyl radical scavenging activity

The results showed that methanolic extracts displayed potential inhibitory effect of hydroxyl radical scavenging activity. The *N. Arbor-tristis* (85.79%) extract exhibited the maximum activity and *J. adhatoda* exhibited the minimum antioxidant activity at the concentration of 500µg/mL.

3.3.3 Superoxide radical scavenging activity

The superoxide radical reduced NBT to blue colored formazan that can be measured at 560 nm. At 10-500µg/mL, the superoxide scavenging activity of the methanolic extract of *O. sanctum* was 26.29-79.31%, and the standard ascorbic acid value was 12.50-71.88%. The result shows that the antioxidant activity of this plant extract is more than the standard ascorbic acid.

3.3.4 Hydrogen peroxide radical scavenging activity

The free radical scavenging activity of the methanolic extract was determined by hydrogen peroxide scavenging assay. From the results, *N. Arbor-tristis* showed the maximum concentration-dependent activity with value 69.19% at the concentration of 500µg/mL (Fig 4).

4. DISCUSSION

Medicinal plants are commonly used to traditionally treat many diseases whose pathogenesis is, among other factors correlated to oxidative stress [3]. However, in order to antioxidant potentials of these plants that could be significant in the treatment of such diseases have not been investigated. Phytochemicals are currently receiving the increased attention of interesting new findings regarding their biological activities. These compounds play some metabolic role and control development in a living system [19]. Phenols are very significant plant constituents because of their radical scavenging ability due to their hydroxyl group. The phenolic contents may contribute directly to the antioxidative action [20]. Presence of phenolic compounds in each plant is varying with its environments. For example, Kabesh *et al.* [21] have reported 178µg gallic acid equivalent/mg plant extract of total phenolic content in *Catharanthes roseus* leaf extract. The highest amounts of flavonoid were found in extracts of *P. nigrum* and other species contained remarkably lower amounts of these compounds. It is well known that plant polyphenols are widely distributed in the plant kingdom and

surprisingly they are present in rich amount. The methanolic extracts were the most effective DPPH radical scavengers. The examination of inhibited DPPH of different plant extract showed different values. The percentages can be considered as a full absorption inhibition of DPPH due to after the whole reaction completed the final solution always possesses some yellowish colour and therefore absorption inhibition compared to colourless methanol can't reach 100% [22]. Hydroxyl radical is the most reactive oxygen-centered species and causes severe damage to adjacent biomolecules [6, 9]. Hydroxyl radical scavenging activity was determined by using hydrogen peroxide – sodium salicylate [23]. Fig. 2 shows that the methanolic extract displayed a potential inhibitory effect of hydroxyl radical scavenging activity. The superoxide anion takes an important role for the configuration of other oxygen species, particularly those are reactive oxygen species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, protein, and DNA [24]. Superoxide anion imitative from dissolved oxygen by PMS-NADH coupling reaction reduces NBT in this system. During the process, superoxide anion reduces the yellow dye (NBT2+) to generate the blue formazan, which is determined spectrophotometrically at 560 nm, and the antioxidants are capable to inhibit the blue NBT formation [25]. Hydrogen peroxide is a weak oxidizing agent and it is not very reactive, can cross biological membranes [26]. Because of the possible involvement of hydrogen peroxide in the generation of hydroxyl radicals, this property places hydrogen peroxide in a more prominent role to initiate cytotoxicity than its chemical reactivity. Thus removing hydrogen peroxide is very important for the protection of living systems [26].

5. CONCLUSION

All the methanolic extracts of plant samples were the strongest radical scavenging activity and it can be used as a natural antioxidant. The further Phytochemical analysis is required to isolate the elements of the plant that show a broad spectrum of pharmacological activity.

REFERENCES

1. Khatun S, Chatterjee NC, Cakilcioglu U. Antioxidant activity of the medicinal plant *Coleus forskohlii* Briq. African Journal of Biotechnology. 2011;10(13):2530-2535.
2. Mahato D, Sahu AP, Sharma HP. Phytochemical and antioxidant evaluation of *Urginea indica* Kunth. Indian Journal of Traditional Knowledge. 2018;17(4):783-788.
3. Harborne JR. Introduction to ecological biochemistry, 4th edn, ((London, Elsevier). 1993.
4. DeLeo FR, Renee J, McCormick S, Nakamura M, Weiss JP, Nauseef WM. Neutrophils exposed to bacterial lipopolysaccharide upregulate NADPH oxidase assembly. Journal of Clinical Investigation. 1998;101:455-463.
5. Nose K. Role of reactive oxygen species in regulation of physiological functions. Biological and Pharmaceutical bulletin. 2000;23:897.

6. Li HB, Wong CC, Cheng KW, Chen F. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. Science Direct. 2008;41:385-390.
7. Govindan P, Muthukrishnan S. Evaluation of total phenolic content and free radical scavenging activity of *Boerhavia erecta*. Journal of Acute Medicine. 2013;3:103-109.
8. Stanner SA, Hugher J, Kelly CN, Butlriss JA, Review of the epidermiological evidence for the antioxidant hypothesis. Public Health and Nutrition. 2000;7:401-422.
9. Pavithra K, Vadivukkarasi S. Evaluation of free radical scavenging activity of various extracts of leaves from *Kedrostis foetidissima.* Science Direct. 2015;4:42-46.
10. Anagnostopoulou MA, Kefalas P, Papageorgious VP, Boskou D. Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*). Food Chemistry.2006; 94:19-25.
11. Sagakami H, Aohi T, Simpson A, Tanuma S. Induction of immunopotentiaion activity by a protein-bound polysaccharide PSK, Anticancer Research, 1991;11:993-1000.
12. Anokwuru CP, Anyasor GN, Ajibaye O, Fakoya O, Okebuhwu P. Effects of Extraction Solvents on Phenolic, Flavonoid and Antioxidant activities of Three Nigerian Medicinal Plants. Nature and Science. 2011;9:53-61.
13. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture*,* 1965; 16:144-153.
14. Quettier DC, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx MC, Cayin JC, Bailleul F, Trotin F. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and Flour. Journal of Ethnopharmacology. 2000;72:35-42.
15. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from Bauhinia terapotensis. Journal of Natural Product. 2001; 64:892-895.
16. Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry*. 1989;28:1057-1060.
17. Liu F, Ooi VE, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. Life Science. 1997;60:763-771.
18. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T, Yoshida T, Okuda T. Effects of the interaction of tannins with co-existing substances. Chemical and Pharmaceutical Bulletin. 1989; 37:2016-2021.
19. Kabesh K, Senthikumar P, Raghunathan R, Raj Kumar R. Phytochemical analysis of Catharanthes roseus plant extract and its antimicrobial activity. International Journal of Pure Applied Bioscience. 2015;3(2):162-172.
20. Nabavi S, Ebrahimzadeh M, Nabavi S, Hamidinia A, Bekhradnia A. Determination of antioxidant activity, phenol and flavonoids content of *Parrotiapersica* Mey. Pharmacology online. 2008;2:560-567.
21. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry. 1999; 269:337-341.
22. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry. 2004;85:231-237.
23. Parejo F, Viladomat J, Bastida, *et al*. Comparison between the radical scavenging activity and antioxidant activity of six distilled and non-distilled Mediterranean herbs and aromatic plants. Journal of Agriculture and Food Chemistry. 2002; 50:6882-6890.
24. Karmakar A, Dolai P, Saha N, Sarkar A, Bala PK. Scavenging activity of *Curcuma caesia* rhizome against reactive oxygen and nitrogen species. Oriental Pharmacy and Experimental Medicine. 2011;11:221-228.

25. Rajamanikandan S, Sindhu T, Durgapriya, D, Sophia D, Ragavendran P, Gopalkrishnan VK. Radical scavenging and antioxidant activity of ethanolic extract of *Mollugo nudicaulis* by *In vitro* assays. Indian Journal of Pharmaceutical Education and Research. 2011: 45:310-316.
26. Van Wijk R, Van Wijk EP, Wiegant FA, Ives J. Free radicals and low-level photon emission in human pathogenesis. State of the art. Indian Journal of Experimental Biology. 2008; 46:273-309.

Fig 1: DPPH free radical scavenging activity of methanolic plant extracts.

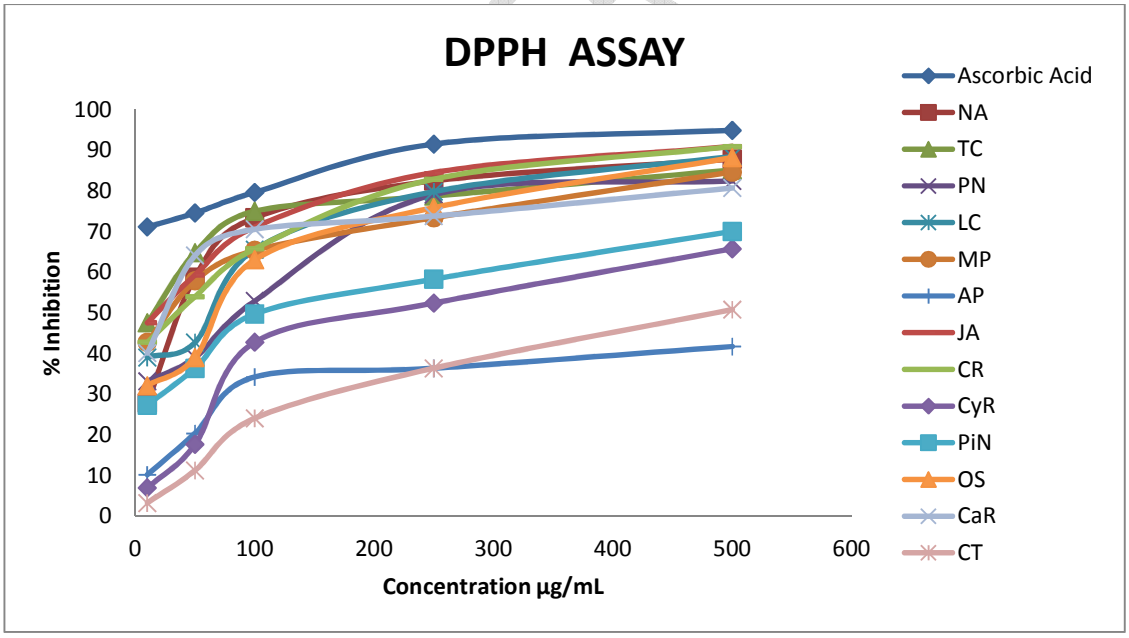

Fig 2: Hydroxyl free radical scavenging activity of methanolic plant extracts.

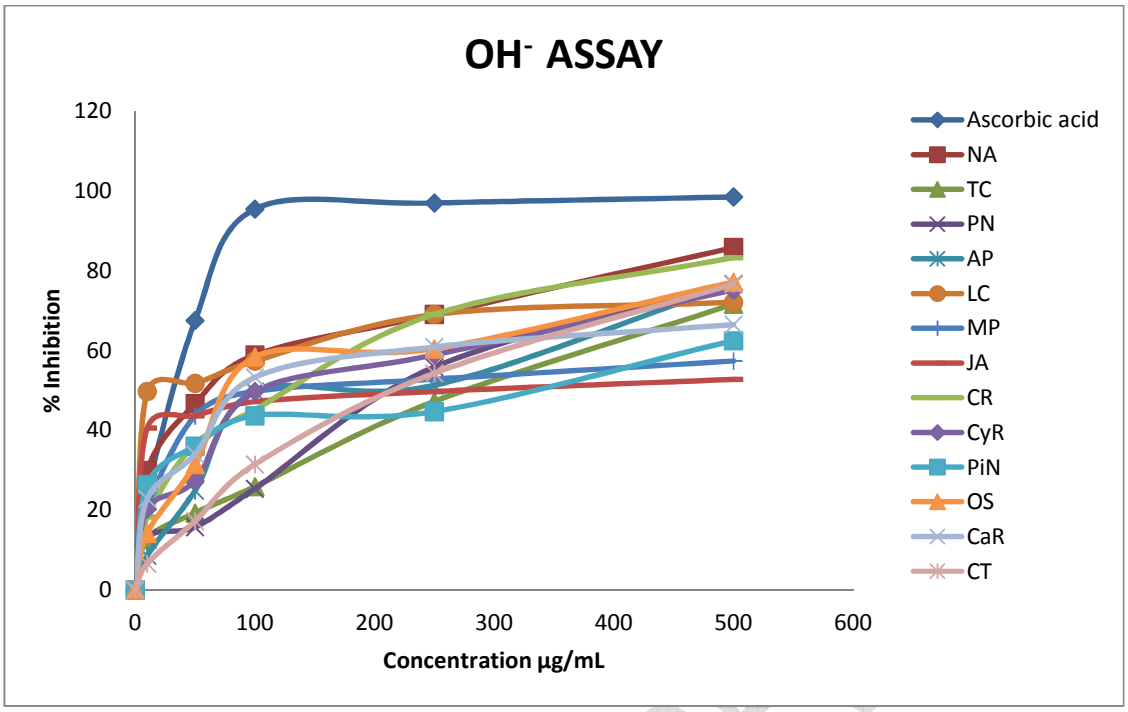

Fig 3: Superoxide free radical scavenging activity of methanolic plant extracts.

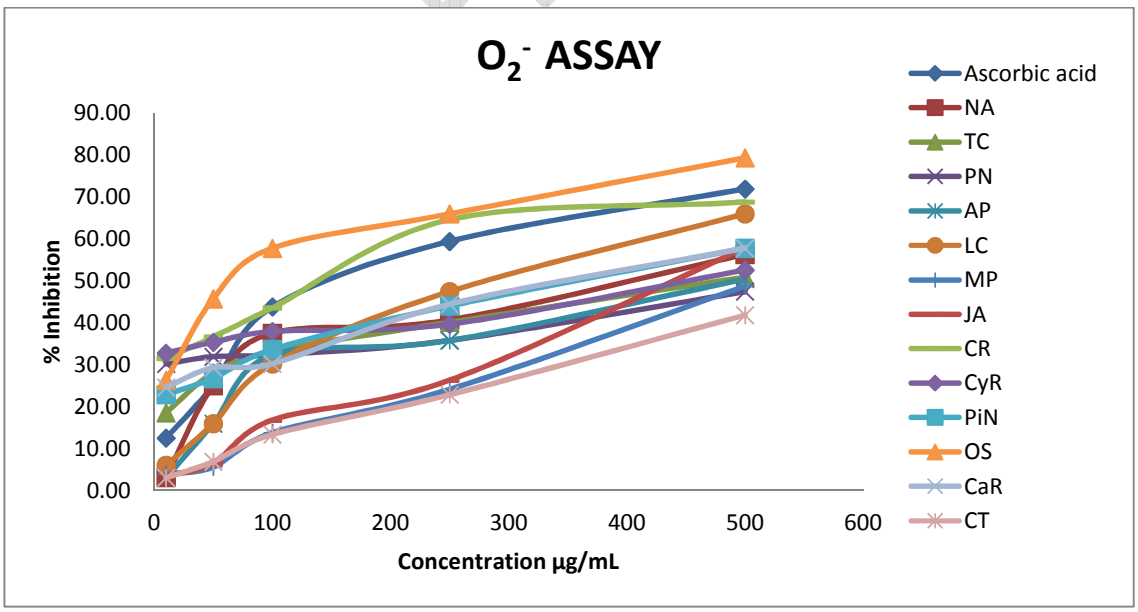

Fig 4: Hydrogen peroxide (H_2O_2) free radical scavenging activity of methanolic plant extracts.

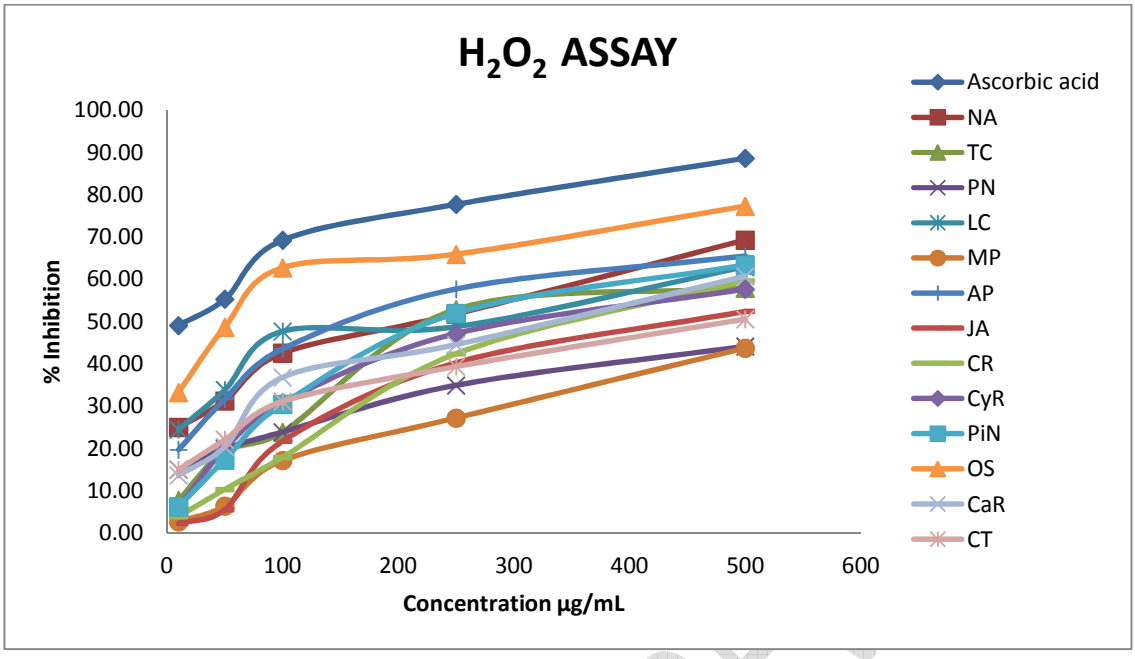

Table 1: Different plant parts used for the antioxidant activity.

Plant species	Family	Parts used
Nyctanthes arbortrisis	Oleaceae	Leaf
Tinospora cordifolia	Menispermaceae	Leaf
Phyllanthes niruri	Euphorbiaceae	Root
Andrographis paniculata	Acanthacae	Leaf
Lantana camara	Verbenaceae	Leaf
Mimosa pudica	Mimosaceae	Root
Justicia adhatoda	Acathaceae	Leaf
Cuscuta reflexa	Convovulaceae	Stem
Cyperus rotendus	Poaceae	Root
Piper nigrum	Pinaceae	Seed
Ocimum sanctum	Lamiaceae	Leaf
Cantharanthus roseus	Apocyanaceae	Leaf

Clitoria ternatea	Fabaceae	Flower

Table 2: Percentage of yield and the total amount of plant phenolic and flavonoid contents.

Plant extracts	**% of Yield**	**Total phenolic compounds mg/g plant extract (in GAE)**	**Total flavonoids mg/g plant extract (in RE)**
Nyctanthes arbortrisis	30.85	106.92 ± 0.18	8.44 ± 0.04
Tinospora cordifolia	24.80	294.07 ± 0.37	25.34 ± 0.10
Phyllanthes niruri	36.08	105.03 ± 0.14	85.34 ± 0.25
Andrographis paniculata	36.40	114.48 ± 0.22	7.24 ± 0.02
Lantana camara	27.20	80.45 ± 0.11	12.34 ± 0.04
Mimosa pudica	27.40	44.53 ± 0.05	11.02 ± 0.05
Justicia adhatoda	27.90	71.00 ± 0.09	21.51 ± 0.05
Cuscuta reflexa	25.40	131.49 ± 0.28	12.97 ± 0.03
Cyperus rotendus	25.50	93.69 ± 0.13	28.27 ± 0.05
Piper nigrum	30.80	120.15 ± 0.25	17.32 ± 0.04
Ocimum sanctum	37.05	137.17 ± 0.27	16.86 ± 0.04
Cantharanthus roseus	24.40	88.01 ± 0.17	5.63 ± 0.03
Clitoria ternatea	38.00	46.43 ± 0.07	22.71 ± 0.07