IJPSR (2019), Volume 10, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 March 2019; received in revised form, 23 June 2019; accepted, 14 July 2019; published 01 December 2019

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF AERIAL PART EXTRACTS OF *CYCLEA PELTATA*

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Keywords:

Antioxidant, DPPH, Free radical scavenging, Hydroxyl radical scavenging, *Cyclea peltata*

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ABSTRACT: In recent years, there is an increasing interest in finding antioxidant phytochemicals for the prevention and treatment of complex diseases like diabetes, cancer, and atherosclerosis, which are associated with excessive biological oxidative stress. Plants are rich source of natural antioxidants that inhibit the propagation of free radical reactions and protect the human body from degenerative diseases. For the present study, entire vine (aerial part) of *Cyclea peltata* was selected based on their extensive use in folk medicine and ayurvedic medicine to cure various ailments. Hexane, chloroform, ethyl acetate, methanol and water extracts of *Cyclea peltata* were subjected to various *in-vitro* antioxidant activity studies. In most of the antioxidant parameters, ethyl acetate and methanol extracts showed potent activity and chloroform extract showed minimum antioxidant activity. From these studies, it can be concluded that ethyl acetate and methanol extracts of *Cyclea peltata* could be used as a source of potent natural antioxidant that can be used in various plant-based drugs or formulations for the treatment of complex diseases caused due to excessive biological oxidative stress.

INTRODUCTION: Oxidation and production of free radicals are an integral part of normal cell metabolism, but become deleterious when not being eliminated by the endogenous defense system. An imbalance between reactive oxygen species and the antioxidant defense mechanisms of a cell leads to excessive production of oxygen metabolites, creating a condition frequently termed as 'Oxidative Stress' [1]. Excessive oxidative stress has been implicated in the pathology and complications of diabetes mellitus [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(12).5416-22

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(12).5416-22

There are increasing evidence to suggest that the free radicals induce oxidative damage of proteins, DNA and lipids, which are also associated with chronic degenerative diseases *viz.*, cancer [3], Cardiovascular diseases [4] and ulcerative colitis [5]. Oxygen-derived free radicals such as superoxide, anions, hydroxyl radicals, and hydrogen peroxide are cytotoxic and give rise to tissue injuries caused due to cellular dysfunction or cell death. *Diabetes mellitus* and its associated complications are one such disorder which may attribute to excessive biological oxidative stress [6].

India has an ancient heritage of traditional medicine. WHO estimates that about 80% of people living in developing countries rely almost exclusively on traditional medicine [7]. Herbal medicines have been the basis of treatment and cure for various diseases and physiological

conditions in traditional methods practiced such as Ayurveda, Unani, Siddha, and folk medicine [8]. Plant phenolics, in particular, phenolic acids, tannins, and flavonoids are known to be potent antioxidants and occur in vegetables, fruits, nuts, seeds, roots, and barks. In addition to their antioxidant properties, these compounds display a wide variety of pharmacological activities such as anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial or anti-viral activities, which may explain, at least in part, its use as an alternative or supportive treatments in various degenerative diseases [9].

In recent years, there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, protect the human body from diseases [10]. Antioxidant-based drugs or formulations for the prevention and treatment of complex diseases like diabetes, cancer, and atherosclerosis have appeared during the last three decades [11]. This has attracted a great deal of research interest in natural antioxidants. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins [12].

Cyclea peltata plant parts were reported for various biological properties such as extracts of *Cyclea peltata* root showed antimicrobial activity [13], extracts of root showed hepatoprotective and antioxidant properties [14], extracts of root showed potent antisecretory and antiulcer effects [15], extracts of root showed antioxidant and antiulcer activity [16]. Extract of the whole plant showed antibacterial activity [17] and extract of leaves possessed significant diuretic activity [18]. However, there is not much work related to antioxidant on whole aerial part (stem and leaves together) of *Cyclea peltata* and hence for the present study entire aerial part of *Cyclea peltata* (*i.e.* stem and leaves together) was selected based on its literature reviews and its extensive use for curing various ailments in folk and ayurvedic system of medicine.

Cyclea peltata (Lam.) Hook. f. & Thomson. That belongs to family Menispermaceae is a slender twining shrub [19] commonly known as Patha or Rajapatha in Sanskrit and Kariballi in Kannada is found throughout South and East India. Tribal people use this plant for treating muscular sprain and wounds. In Ayurvedic system of medicine, tuberous roots are being used in the treatment of urinary troubles [20], asthma[21], jaundice [21], fever [21] and stomachache [22].

Given this, the present study aims at the investigation of phytochemical screening and antioxidant activities of *Cyclea peltata* (aerial part) extracts. These investigations may even become the base for the development of antioxidant-based herbal drugs or formulations for treating complex diseases and thereby usage of synthetic drugs; its associated side effects could be avoided.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material:

Plant material of *Cyclea peltata* was collected from rural areas of Mysuru district, Karnataka, India, during July-August 2014. Plant identified and authenticated by Botanist Dr. M. N. Naganandini, Asst. Professor, JSS College of Pharmacy, Mysuru.

The herbarium specimen was made and was deposited in the Department of Pharmacognosy, JSS College of Pharmacy, Mysuru with voucher specimen number *Cyclea peltata* JSSCP/PCOG/ HRBM-06. For the study, only the aerial part of *Cyclea peltata* (*i.e.*, leaves and stem together) was selected. The fresh plant material was washed under running tap water to remove the any adhered dust and shade dried in room temperature for 7 days and ground to a coarse powder of mesh size 40-60 and stored in an airtight container till use.

Extraction Procedure: Extraction was carried out by cold maceration method with different solvents *viz.* hexane, chloroform, ethyl acetate, methanol, and water. 500g of shade-dried powder of plant material was kept in a closed glass container for 7 days at room temperature. The macerate was first filtered through a double-layered muslin cloth and then filtered through Whatman no.1 filter paper to get clear filtrate (free from suspended material). The filtrate (filtrates of all extracts except water extract) was concentrated to thick syrupy mass using Flash Rotary Evaporator and dried on a water bath. The dried extracted was kept in a desiccator over anhydrous Sodium sulfate for further drying and preserved in a brown bottle at 4 ºC until further use. Whereas water extracts were concentrated to

thick and dried on the water bath, kept in a desiccator over anhydrous Sodium sulfate for further drying. The dried extract was preserved in a brown bottle at 4ºC until further use [7, 23].

Phytochemical Screening: Phytochemicals are the wide variety of secondary metabolites produced by plants. These are called as the active chemical constituents of plants. They are tannins, alkaloids, saponins, flavonoids, phytosterols, triterpenoids, and cardiac glycosides. The Presence of these active chemical constituents in plant extracts are determined by preliminary qualitative phytochemical screening according to the procedures described by Mukherjee [7], Evans [24,] and Harborne [25].

***In-vitro* Antioxidant Activity Studies of the Plant Extracts:** Antioxidants are the molecules or substances which prevent the oxidative damage caused by free radicals in our body. Hence, they are called "free radical scavengers." Antioxidants inhibit oxidation reactions caused by free radicals by being oxidized themselves, so antioxidants are often reducing agents, and they act as adjuvant. Antioxidant activity of plant extracts was carried out by the following methods**.**

DPPH Radical Scavenging Activity: Free radical scavenging potentials of the extracts were tested against the methanolic solution of α-α-diphenyl-β-picrylhydrazyl (DPPH) [9]. Different concentrations of various extracts and ascorbic acid were taken in 1ml ethanol and added to 3 ml of 100 µM DPPH in methanol to get the concentration of extracts from 5 µg/ml to 800 µg/ml and ascorbic acid from 1.25 µg/ml to 5 µg/ml. The mixture was allowed to stand at room temperature for 20 min. The control was prepared as above without extract. The readings were read at 517 nm using methanol as blank. The absorbance of control was first noted at 517 nm. The change in absorbance of the samples was measured. Scavenging activity was expressed as % Antiradical activity calculated by the formula {(Control Abs. - Sample Abs.) / Control Abs.} × 100. Each experiment was carried out in triplicate, and the results are expressed as mean % antiradical activity ± SD [9].

Nitric Oxide Scavenging Activity: Sodium nitroprusside (SNP 10 mM) in phosphate buffered saline was mixed with different concentrations of CpA extract [Hexane, Chloroform, Ethyl acetate, Methanol and Aqueous extracts of aerial part of *Cyclea peltata*] (10 µg/ml to 600 µg/ml) in ethanol and incubated at 25 ºC for 180 min. Ascorbic acid, instead of the extract was used as a positive control in the concentration of 25 µg/ml to 125 µg/ml.

Further on, an equal volume of the Griess reagent (1% sulfanilamide, 0.1% naphylethylenediamine dihydrochloride and 3% phosphoric acid) was added to the incubated solution. The absorbance was immediately measured at 546 nm [9]. Nitric oxide scavenging activity was calculated with the following equation: Nitric oxide scavenging activity = {(ABS of Control - ABS of the sample) / ABS of Control} × 100. All experiments were performed in triplicate. The results are expressed as mean % NO scavenging activity ± SD [9].

Hydroxyl Radical Scavenging Activity: The mixture containing CpA extract [Hexane, Chloroform, Ethyl acetate, Methanol and Aqueous extracts of aerial part of *Cyclea peltata*] (10 µg to 80 µg), 2.8 mM deoxy-Dals- Ribose, and 0.2 mM phenylhydrazine was incubated for 2 hours at 37^0C in a water bath. Gallic acid, was employed as a positive control (0.5 µg to 4 µg). Hydroxyl radical scavenging was measured by the TBARS method of Ohkawa *et al.* [26] To each test tube, 1 ml of TCA (2.8%, % w/v), containing 1% TBA, was added. Further, the test tubes were heated in a water bath for 30 min and cooled at room temperature. A mixture prepared as above without deoxy-D- ribose served as blank. Absorbance was read at 532 nm. Hydroxyl radical scavenging activity was calculated with the following equation: percentage hydroxyl radical scavenging activity = {(C- S)/C} × 100, Where C is the absorbance of the control and S is the absorbance of the sample. All experiments were performed in triplicate, and the results are expressed as mean ± SD [26].

Lipid Peroxidation Assay: Egg phospatidyl-choline (20 mg) in chloroform (2 ml) was dried under vacuum in a rotary evaporator to give a thin homogenous film and further dispersed in normal saline (5 ml) with a vortex mixer. The mixture was sonicated to get a homogeneous suspension of liposomes. Lipid peroxidation was initiated by adding 0.05 mM ascorbic acid into a mixture containing liposome (0.1ml), 150 mM potassium

chloride, 0.2 mM ferric chloride, CpA extract [Hexane, Chloroform, Ethyl acetate, Methanol and Aqueous extracts of aerial part of *Cyclea peltata*] (10 to 80 µg/ml), or silymarin (1 to 10 µg) in a total volume of 1 ml [27].

The reaction mixture as incubated at 37 °C for 40 min. After incubation, the reaction was stopped by adding 1 ml of ice-cold 0.25 M sodium hydroxide containing 20% TCA (w/v), 0.4% TBA (w/v), and 0.05% BHT (w/v). After incubating in a boiling water bath for 20 min, the samples were cooled to room temperature.

The blank was prepared in the same manner but, without liposomes. The pink chromogen was extracted with 1 ml of n-butanol. The absorbance was read at 532 nm. Anti-lipid peroxidation activity was calculated with the following equation: % Anti-lipid peroxidation activity = {(C- S)/C} × 100, Where C is the absorbance of the control and S is the absorbance of the sample. All experiments were carried out in triplicate, and results are expressed as mean ± SD [27].

RESULTS AND DISCUSSION:

TABLE 1: PHYTOCHEMICAL STUDIES OF *CYCLEA PELTATA* EXTRACTS

Phytochemicals	Extracts				
	CPH	CPC	CPEA	CPM	CPW
Sterols	+	+	+	+	-
Triterpenes	-	-	-	-	-
Alkaloids	-	+	+	+	-
Glycosides	-	-	-	+	+
Saponins	-	-	-	+	+
Flavonoids	-	-	+	+	+
Phenolics & Tannins	-	-	+	+	+
Carbohydrates	-	-	-	+	+

Note: *Cyclea peltata* aerial part- Hexane, Chloroform, Ethyl acetate, Methanol and aqueous extracts- (CPH, CPC, CPEA, CPM & CPW); + = present, - = absent

Table 1 shows that Hexane extract of *Cyclea peltata* found to contain only sterols, chloroform extract found to contain sterols and alkaloids, Ethyl acetate extract found to contain sterols, alkaloids, flavonoids, phenolics. Methanol and aqueous extracts found to contain all the constituents *viz*, sterols, alkaloids, glycosides, saponins, flavonoids, and phenolics.

TABLE 2: OH RADICAL SCAVENGING ACTIVITY OF *CYCLEA PELTATA* EXTRACTS

Conc. in µg/ml	CPH	CPC	CPEA	CPM	CPW
10	0.91 ± 1.57	18.15 ± 1.98	19.77 ± 2.40	11.56 ± 2.25	13.14 ± 1.23
20	2.90 ± 2.64	32.48 ± 2.42	29.48 ± 1.80	18.90 ± 1.59	20.98 ± 2.51
40	12.32 ± 1.26	41.12 ± 1.57	47.74 ± 3.23	33.84 ± 1.73	31.67 ± 1.15
60	29.72 ± 1.35	52.20 ± 3.47	82.33 ± 2.24	78.96 ± 2.20	44.08 ± 1.42
80	34.05 ± 3.52	62.11 ± 1.25	88.37 ± 2.15	88.36 ± 2.35	59.72 ± 2.22
IC_{50} in µg/ml	ND	57	42	47	69

TABLE 3: OH RADICAL SCAVENGING ACTIVITY OF GALLIC ACID

Conc. in µg/mL	Gallic acid
0.5	19.07 ± 4.23
1	26.72 ± 6.47
2	35.39 ± 2.41
3	46.39 ± 3.92
4	57.89 ± 6.17
IC_{50} in µg/ml	3.35

Table 2 reveals that Potent OH radical scavenging activity is exhibited by CPEA with IC_{50} values of 42 µg/ml followed by CPM with IC_{50} values of 47 µg/ml. CPH has shown least OH radical activity has shown 34.05% at 80 µg/ml, which is the highest concentration tested. The OH radical scavenging activity from lower to higher was found in the following order CPH<CPW<CPC<CPM< CPEA.

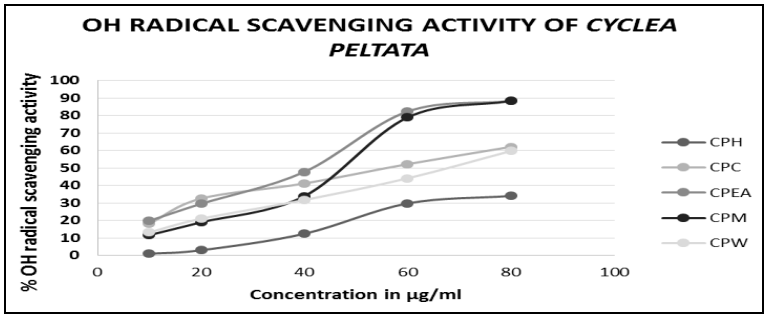

FIG. 1: OH RADICAL SCAVENGING POTENTIAL OF CYCLEA PELTATA EXTRACTS

TABLE 4: NITRIC OXIDE SCAVENGING POTENTIAL OF *CYCLEA PELTATA* EXTRACTS

Conc. in µg/ml	CPH	CPC	CPEA	CPM	CPW
10	15.48 ± 2.20	20.97 ± 1.58	18.31 ± 1.78	20.75 ± 3.15	22.30 ± 1.23
25	35.61 ± 1.23	44.46 ± 1.34	36.50 ± 1.65	45.13 ± 1.12	38.49 ± 1.44
50	40.04 ± 1.15	45.35 ± 2.21	48.67 ± 3.56	47.78 ± 1.17	44.91 ± 2.78
100	45.13 ± 1.34	52.43 ± 1.15	53.31 ± 1.34	53.09 ± 1.45	52.65 ± 1.13
200	52.43 ± 2.27	59.73 ± 1.40	60.84 ± 1.41	54.20 ± 2.20	61.28 ± 1.35
400	58.84 ± 1.64	66.59 ± 2.76	68.14 ± 1.22	61.94 ± 2.57	69.24 ± 3.21
600	70.62 ± 3.26	76.10 ± 2.32	71.90 ± 2.56	73.00 ± 1.41	76.99 ± 1.52
IC_{50} µg/ml	152.5	80.5	55.0	62.5	70.0

TABLE 5: NITRIC OXIDE SCAVENGING ACTIVITY OF ASCORBIC ACID

Conc. in µg/ml	% NO. Scavenging activity
25	35.45 ± 6.22
50	43.67 ± 3.47
75	44.92 ± 6.04
100	60.15 ± 4.37
125	71.66 ± 4.68
IC_{50} in µg/ml	84.16

Table 4 reveals that Potent NO scavenging activity is exhibited by CPEA with IC_{50} values of 55 µg/ml, followed by CPM with IC_{50} values of 62.5 µg/ml. CPH has shown least NO scavenging activity. The NO radical scavenging activity was found from lower to higher in the following order: CPH < CPC < CPW < CPM < CPEA.

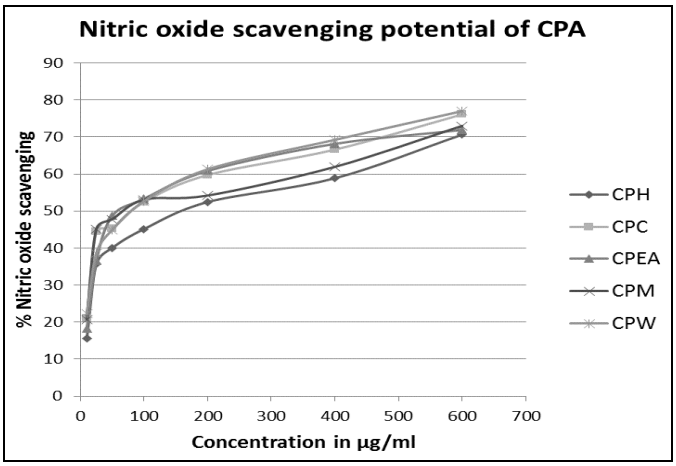

FIG. 2: NITRIC OXIDE SCAVENGING POTENTIAL OF *CYCLEA PELTATA* EXTRACTS

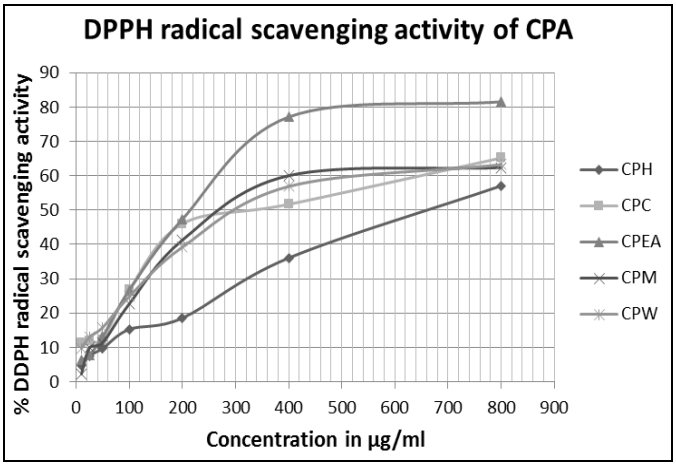

FIG. 3: DPPH RADICAL SCAVENGING ACTIVITY OF *CYCLEA PELTATA* EXTRACTS

TABLE 6: ANTIOXIDANT ACTIVITY STUDY OF *CYCLEA PELTATA* EXTRACTS BY DPPH METHOD

Conc. in µg/ml	CPH	CPC	CPEA	CPM	CPW
10	4.68 ± 3.14	11.22 ± 1.22	6.36 ± 1.26	2.32 ± 1.34	9.72 ± 1.84
25	7.60 ± 1.82	11.93 ± 1.64	7.77 ± 1.71	9.89 ± 1.56	13.07 ± 1.60
50	9.72 ± 1.73	12.19 ± 3.59	13.16 ± 1.53	11.40 ± 3.25	15.81 ± 2.10
100	15.28 ± 1.15	26.86 ± 2.23	26.68 ± 2.86	22.70 ± 1.16	24.82 ± 1.92
200	18.55 ± 2.21	46.02 ± 1.14	47.35 ± 1.78	41.27 ± 1.28	39.22 ± 1.33
400	36.04 ± 1.19	51.68 ± 1.72	77.12 ± 1.11	60.04 ± 1.46	56.89 ± 3.21
800	57.07 ± 1.35	65.19 ± 1.46	81.54 ± 1.57	62.38 ± 2.42	63.25 ± 1.24
IC_{50} in µg/ml	658	304	212	257	290

TABLE 7: DPPH RADICAL SCAVENGING ACTIVITY OF ASCORBIC ACID

Conc. in µg/ml	Ascorbic acid
5	45.38 ± 6.25
10	54.85 ± 1.87
15	89.14 ± 2.26
20	94.81 ± 0.87
25	96.19 ± 2.35
IC_{50} in µg/ml	5.96

Table 6 reveals that Potent DPPH radical scavenging activity is exhibited by CPEA with IC_{50} values of 212 µg/ml followed by CPM with IC_{50} values of 257 µg/ml. Surprisingly CPH has shown potent DPPH radical scavenging activity when compared to, methanol, chloroform, and aqueous extracts.

TABLE 8: ANTIOXIDANT ACTIVITY STUDY OF *CYCLEA PELTATA* EXTRACTS BY LIPID PEROXIDATION ASSAY

Conc. in µg/ml	CPH	CPC	CPEA	CPM	CPW
10	12.38 ± 2.56	20.87 ± 1.33	29.29 ± 1.14	25.63 ± 1.16	60.63 ± 1.63
20	23.81 ± 1.12	54.76 ± 2.27	59.92 ± 2.68	55.16 ± 1.78	71.03 ± 1.22
30	25.79 ± 1.28	55.56 ± 1.56	67.46 ± 1.29	58.33 ± 2.34	75.79 ± 1.87
40	27.78 ± 3.56	57.94 ± 1.32	69.05 ± 1.46	58.73 ± 2.41	79.76 ± 3.43
50	30.16 ± 1.43	58.33 ± 3.45	73.41 ± 1.95	63.49 ± 1.32	80.95 ± 2.29
60	39.68 ± 2.30	58.73 ± 2.11	75.79 ± 2.32	65.48 ± 1.98	81.75 ± 2.66
80	41.27 ± 2.55	60.71 ± 2.20	78.17 ± 1.53	67.46 ± 1.47	82.14 ± 1.26
IC_{50} in µg/ml	ND	19	18.5	19	5

TABLE 9: ANTI-LIPID PEROXIDATION ACTIVITY OF STANDARDS (SILYMARIN)

Conc. in µg/ml	Standard
1	21.36 ± 3.82
2	27.92 ± 1.42
3	38.39 ± 5.63
4	48.16 ± 1.15
5	64.57 ± 3.19
IC_{50} in µg/ml	3.76

Table 8 reveals that Aqueous extract has shown the highest potent lipid peroxidation with an IC_{50} value of 5 µg/ml. CPC, CPEA, and CPM have shown potent lipid peroxidation activity in the same range. CPH has shown the least lipid peroxidation activity with only 41.27 µg/ml at the highest concentration tested 80 µg/ml.

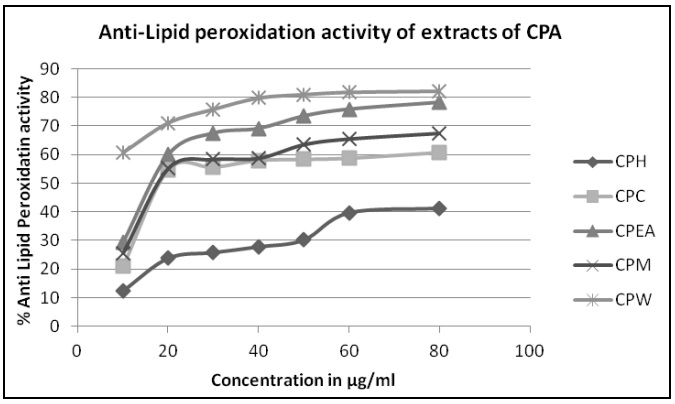

FIG. 4: LIPID PEROXIDATION ACTIVITY OF EXTRACTS OF *CYCLEA PELTATA* EXTRACTS

DISCUSSION: Aerial parts of the plant CP has shown various biological properties. In recent years, the antioxidant activity of the plant sources was attributed to their various biological properties. Also, the antioxidant activity of the extracts has become an important parameter of standardization as the methods of determining antioxidant activity are easier, reproducible, and reliable. Also, the roots of the plant were found to exhibit potent antioxidant and hepatoprotective activity. Because of all the above reasons, the study was undertaken to evaluate the antioxidant activity of aerial parts of CP.

As the phytochemical screening revealed, hexane extract found to contain sterols & ethyl acetate extracts found; chloroform extract found to contain, sterols and alkaloids; methanol extract found to contain all the above constituents. Water extract found to contain glycosides, carbohydrates, phenolics. It is a known factor that phenolics (flavonoids, tannins, phenolic acids) are potent antioxidants. The aerial part of CP found to contain phenolics as per the phytochemicals tests carried out and are the reason for the potent antioxidant potential of CP. It is also evident that the ethyl acetate extract has shown highly potent antioxidant activity owing to the presence of phenolics in the extract. Other constituents present in the extract were sterols and alkaloids. Ethyl acetate is the choice of the solvent for extracting flavonoids. So, it can be understood that ethyl acetate extracted most of the flavonoids and other phenolics. Flavonoids and phenolics were reported to be potent antioxidants, and so is the activity of CPEA.

The antioxidant activity of different extracts in all the parameters tested *viz.* DPPH radical scavenging activity, OH radical scavenging activity, NO oxide scavenging activity, and Lipid peroxidation activity were found in the order from lower to higher in the order CPH < CPC < CPW < CPM < CPEA. *i.e.*, hexane extract showed least antioxidant potential, and ethyl acetate extract exhibited potent antioxidant potential. CPM (methanol extract) which has shown better antioxidant potential other than water and chloroform extracts, but less than the CPEA (ethyl acetate extract). The reason for this may be, as per the phytochemical screening, methanol being a polar solvent extracted all types of constituents present in the plant material, *i.e.* CPM contains sterols, alkaloids, glycosides, and carbohydrates, which are deemed to be antioxidants, whereas CPEA not extracted glycosides and carbohydrates.

So, the percentage of flavonoids and phenolics were more in CPEA and so the potential antioxidant activity. In similar lines, water extract extracted all but not sterols and alkaloids. So, aqueous extract (CPW) was shown a potent antioxidant activity.

CONCLUSION: In most of the antioxidant parameters ethyl acetate and methanol extracts showed potent activity, and chloroform extract showed minimum antioxidant activity. From these studies, it can be concluded that ethyl acetate and methanol extracts of *Cyclea peltata* could be used as a source of potent natural antioxidant that can be used in various plant-based drugs or formulations for the treatment of complex diseases caused due to excessive biological oxidative stress.

ACKNOWLEDGEMENT: The authors are thankful to, The Chairperson, Department of Microbiology, University of Mysore, Manasagangotri, Mysuru for providing necessary infrastructure and facilities carry out the research.

CONFLICTS OF INTEREST: The authors are declared that there is no conflicts of interest exist.

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How to cite this article:
Jagadeepchandra S, Mruthunjaya K and Lakshmidevi N: Phytochemical screening and antioxidant activity of aerial part extracts of *Cyclea peltata*. Int J Pharm Sci & Res 2019; 10(12): 5416-22. doi: 10.13040/IJPSR.0975-8232.10(12).5416-22.