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Phenolic Contents and Antioxidant Properties from Aerial Parts of *Achyranthes coynei* Sant

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Aim of the study was to evaluate antioxidant activity and total phenolic content of *Achyranthes coynei;* an endemic plant used in treatment of several diseases in the same lines that of *Achyranthes aspera* by traditional practitioners of Belgaum region. Efficiency of extraction methods was studied for aerial parts (leaves, stem, and inflorescence) extracted in methanol using continuous shaking, microwave assisted and ultra sonic extraction technique, by exposing it for different time period. Total phenolic content was measured by Folin-Ciocalteu method and antioxidant activity using 2,2'-diphenyl-1-picryl hydrazyl radical scavenging assay and ferric reducing antioxidant power assay. Extracts of *A. coynei* revealed highest yield of total phenolic content in continuous shaking method compared to other methods. Significantly higher amount of phenolic content (467.07±23.35 tannic acid equivalent and 360.83±18.04 caffic acid equivalent mg/100 g FW) was estimated at 360 min of continuous shaking extraction. In 2,2'-diphenyl-1-picryl hydrazyl radical scavenging assay and ferric reducing antioxidant power assay, inflorescence and leaf showed highest potential activity, respectively. Stem extracts showed lower yield of total phenolic content and antioxidant activity. Results also showed 2,2'-diphenyl-1-picryl hydrazyl radical scavenging assay had significant correlation with total phenolic content. This is first report of total phenolic content and antioxidant studies in *A. coynei.*

Key words: Antioxidant activity, *Achyranthes coynei,* endemic, total phenolic content

Plants are good source of biologically active secondary metabolites which have many therapeutic potential in many diseases and even in free radical associated disorders[1]. Among secondary metabolites synthesized, plant polyphenols are the aromatic hydroxylated compounds which have the most potent and therapeutically useful bioactive substances. Promising radical scavenging ability of the phenolic compounds produced in higher plants is studied extensively[2,3]. Oxidation stress is one of the major concerns of health in modern era and antioxidants have been reported to prevent oxidative damage caused by free radical, via interfering with the oxidation process by reacting with free radicals, by chelating with catalytic metals, and also by acting as oxygen scavengers[4,5]. Although several synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, are available because of their toxicity problems; there is an upsurge of interest in the therapeutic potentials of plants as antioxidants. In addition to the natural antioxidants like vegetables, fruits, spices and tea, scientific evaluation of plant's properties through potent pharmacological activities, toxicity profiling and economic viability are needed for growing recognition for medicinal plants and herbal products as novel antioxidants in recent decades. Therefore, significant consideration has been directed toward the detection of antioxidant properties in plant species.

Achyranthes coynei Sant. is a rare, endemic plant species belonging to family Amaranthaceae. Its distribution was restricted to Maharashtra and recently was reported from Karnataka[6]. *Achyranthes aspera* L. is the much known medicinal plant from the family used in treating various disorders[7]. *Achyranthes coynei*, locally known as "*Kempu uttarani*" is used as substitute for *A. aspera* by local traditional practitioners in similar disease treatment because of comparable appearance[6].

Green leaves, stem, and inflorescences of *A. coynei* were obtained from a single produce at Pachapur,

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from Belgaum and a specimen was authenticated and deposited at Herbaria, Regional Medical Research Centre, Belgaum (Voucher Number: RMRC-785). Three extraction methods with three exposure times were compared for their antioxidant activity and the yield of phenolics. For all methods 1 g of plant materials (green leaves, stem, and inflorescence) were extracted with 20 ml of 95% methanol. Continuous shaking extraction (CSE) was carried out on orbital shaker (Remi Instruments, Mumbai, India) at a constant stirring of 110 rpm at room temperature for 30, 180, and 360 min separately. Microwave assisted extraction (MAE) was carried out at 1, 3, and 5 min of exposure using microwave oven (Godrej, GM×30 CA1 SIM) at 180 W. The suspensions were cooled after every 1 min to avoid bumping of solvent out of the flask in order to complete 3 and 5 min of microwave exposure. Ultrasonic extraction (USE) was performed on ultrasonic bath (Soncis Vibracell, USA) 130 Watt, at working amplitude of 60 Khz. The samples were exposed for 5, 15, and 30 min of sonication at room temperature. These extracts were filtered using Watman filter paper No. 1 and volume was made up to 20 ml with 95% methanol.

Total phenolic content (TPC) was quantified using modified Folin–Ciocalteu method described by Wolfe *et al.*[8]. The absorbance of blue color was read at 760 nm on double beam spectrophotometer. The results were compared to the standard curve and were expressed as mg tannic and/or caffeic acid equivalent per 100 g fresh plant material.

Antioxidant activities were determined as the measure of free radical scavenging activity using 2,2'-diphenyl-1-picryl hydrazyl (DPPH) assay as determined by Brand-Williams *et al.*[9]. The absorbance at 515 nm was measured using methanol as blank and DDPH radical scavenging activity was calculated. Ferric reducing antioxidant power (FRAP) assay was used to measure the total antioxidant power. Antioxidant assay was preformed as previously described and absorbance was taken at 593 nm[10]. The results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC) and Trolox equivalent antioxidant capacity (TEAC) as determined by Gil *et al.*[11].

The values are represented as mean±SD of three individual readings. The calibration curves for all standards were generated with the correlation coefficients (R^2) above 0.9500. The regression equations for various determinations are given as follows, for TPC: tannic acid, y=0.003x+0.115; caffeic acid y=0.004x+0.092; for DPPH assay: TEAC, y=0.000x+0.001; AEAC, y=0.000x-0.024; for FRAP assay: TEAC, y=0.000x+0.152; AEAC, y=0.000x+0.114. The respective contents were calculated using above equations from standard calibration curves.

Estimated TPC from various parts of *A. coynei* extracted using CSE, MAE, and USE methods are depicted in Table 1. Total phenolic content ranged from 85.13±4.26 to 467.07±23.35 mg tannic acid equivalent (TAE)/100 g and 65.77±03.29 to 360.83±18.04 mg caffeic acid equivalent (CAE)/100 g on fresh weight basis. TPC in stem was lowest from 85.13±4.26 mg TAE/100 g and 65.77±3.29 mg CAE/100 g at 30 min to highest of 190.02±9.50 mg TAE/100 g and 146.80±7.34 mg CAE/100 g at 360 min of CSE. TPC in leaves ranged from 220.79±11.04 to 354.51±17.73 mg TAE/100g and

TABLE 1: EFFICIENCY OF EXTRACTION METHODS ON CONTENT OF TOTAL PHENOLICS IN VARIOUS PARTS OF *A. COYNEI*

Method of extraction	Plant parts	Time min	Total phenolic content mg/100g FW	
			Tannic acid±sd	Caffeic acid±sd
Continuous shaking extraction	Leaf	30	312.67±15.63	241.56±12.08
		180	354.51±17.73	273.88±13.69
		360	315.59±15.78	243.81±12.19
	Stem	30	086.10±04.31	066.52±03.33
		180	142.35±07.12	109.98±05.50
		360	190.02±09.50	146.80±07.34
	Inflorescence	30	302.90±15.15	234.01±11.70
		180	368.44±18.42	284.64±14.23
		360	467.07±23.35	360.83±18.04
Microwave assisted extraction	Leaf	1	295.94±14.80	228.63±11.43
		3	270.08±13.50	208.65±10.43
		5	324.39±16.22	250.61±12.53
	Stem	1	115.90±05.80	089.54±04.48
		3	130.15±06.51	100.55±05.03
		5	145.43±07.27	112.35±05.62
	Inflorescence	1	250.32±12.52	193.39±09.67
		3	411.14±20.56	317.63±15.88
		5	249.67±12.48	192.89±09.64
Ultra sonic extraction	Leaf	5	220.79±11.04	170.57±08.53
		15	290.65±14.53	224.54±11.23
		30	300.42±15.02	232.09±11.60
	Stem	5	085.13±04.26	065.77±03.29
		15	107.27±05.36	082.87±04.14
		30	101.38±05.07	078.32±03.92
	Inflorescence	5	297.23±14.86	229.63±11.48
		15	303.17±15.16	234.22±11.71
		30	364.98±18.25	281.97±14.10

SD=Standard deviation

170.57±8.53 to 273.88±13.69 mg CAE/100g. Among all parts and extraction methods tested highest TPC was observed in inflorescence (467.07±23.35 TAE and 360.83±18.04 CAE mg/100 g) at 360 min of CSE method and lowest was recorded in stem. Synchronized patterns of increase in TPC with respect to time of extraction were observed for inflorescence in CSE and USE; stem in CSE and MAE and leaf in MAE and USE methods.

Antioxidant potential of aerial parts of *A. coynei* using different extraction methods were tested using DPPH and FRAP assay and results were presented in Table 2. Ascorbic acid equivalent activity was recorded higher over trolox in both antioxidant assays for the tested extracts. The DDPH radical scavenging activity was highest in inflorescence extract with CES 360 min (TEAC 473.63 μM and AEAC 666.43 μM) as per Table 2. The results were in correlation to the phenolic content estimated. Increase in DPPH activity with respect to time (30-360 min) was observed in CSE and MAE methods. Minor fluctuation in the activity was observed for extracts exposed to USE method. However, it was interesting to note that in CSE yielded higher and significant results over the other methods tested.

Ferric reducing capacity was higher in leaf sample extracted using 180 min of CSE method (688.82±34.44 μM TEAC and 844.02±42.20 μM AEAC). The pattern observed for the FRAP activity was unlike that of DPPH and TPC. Increase in activity was proportional to the immediate next exposure time in series for all the extraction methods followed by drop or no change (Table 2). This may be because extended exposure time in any extraction method affecting activity. Small variation in activity was noticed with change in exposure times for a particular method and plant part. Leaf yielded highest activity for FRAP over stem and inflorescence.

Different levels reported in this study may be attributed to the different plant parts and extraction methods with time used to express as total phenolic contents. In general, there was a significant correlation between TPC and DPPH scavenging assays over FRAP. Findings of the study also indicate polyphenols are important contributors in free radical scavenging

TABLE 2: EFFICIENCY OF EXTRACTION METHODS ON DPPH RADICAL SCAVENGING AND FRAP ANTIOXIDANT ACTIVITIES IN VARIOUS PARTS OF *A. COYNEI*

Method of extraction	Plant parts	Time min	DPPH μM		FRAP μM	
			TEAC±SD	AEAC±SD	TEAC±SD	AEAC±SD
Continuous shaking extraction	Leaf	30	239.17±11.96	337.04±16.85	462.14±23.11	566.26±28.31
		180	386.44±19.32	544.57±27.23	688.82±34.44	844.02±42.20
		360	432.39±21.62	609.32±30.47	508.37±25.42	622.91±31.15
	Stem	30	110.16±05.51	155.24±07.76	173.98±08.70	213.18±10.66
		180	208.54±10.43	293.87±14.69	286.28±14.31	350.78±17.54
		360	291.6±14.58	410.92±20.55	293.59±14.68	359.73±17.99
	Inflorescence	30	231.51±11.58	326.25±16.31	254.55±12.73	311.90±15.60
		180	418.84±20.94	590.23±29.51	451.70±22.59	553.47±27.67
		360	473.63±23.68	667.43±33.37	547.82±27.39	671.25±33.56
Microwave assisted extraction	Leaf	1	315.75±15.79	444.96±22.25	491.05±24.55	601.69±30.08
		3	304.56±15.23	429.18±21.46	515.89±25.79	632.12±31.61
		5	374.07±18.70	527.14±26.36	586.44±29.32	718.57±35.93
	Stem	1	169.66±08.48	239.08±11.95	193.81±09.69	237.48±11.87
		3	195.58±09.78	275.61±13.78	239.11±11.96	292.98±14.65
		5	202.06±10.10	284.74±14.24	231.80±11.59	284.03±14.20
	Inflorescence	1	220.91±11.05	311.30±15.57	251.00±12.55	307.56±15.38
		3	353.45±17.67	498.08±24.90	350.26±17.51	429.17±21.46
		5	196.17±09.81	276.44±13.82	228.36±11.42	279.81±13.99
Ultra sonic extraction	Leaf	5	229.74±11.49	323.75±16.19	391.69±19.58	479.94±24.00
		15	284.53±14.23	400.96±20.05	499.61±24.98	612.17±30.61
		30	311.04±15.55	438.31±21.92	493.66±24.68	604.88±30.24
	Stem	5	107.80±05.39	151.92±07.60	286.28±14.31	350.78±17.54
		15	139.61±06.98	196.74±09.84	254.24±12.71	311.52±15.58
		30	132.54±06.63	186.78±09.34	204.87±10.24	251.03±12.55
	Inflorescence	5	237.40±11.87	334.55±16.73	266.55±13.33	326.61±16.33
		15	259.20±12.96	365.26±18.26	295.46±14.77	362.03±18.10
		30	295.13±14.76	415.90±20.80	327.09±16.35	400.78±20.04

SD=Standard deviation, TEAC=Trolox equivalent antioxidant capacity, AEAC=acid equivalent antioxidant capacity, FRAP=ferric reducing antioxidant power, DPPH=diphenyl-1-picryl hydrazyl

activities. The results are in accordance with those carried out in other plants[12].

Hagerman *et al.*[13] reported that the high molecular weight phenolics (tannins) have potent scavenging activity toward the free radicals and that the activity depends on the molecular weight, the number of aromatic rings, and nature of hydroxyl groups. Therefore, antioxidant activities of these extracts cannot be predicted on the basis of their TPC alone, but will also require proper characterization of individual phenolic components.

The present study reports TPC and antioxidant activity of *A. coynei* for the first time. Results showed antioxidant potential of aerial parts using different extraction methods. The study may support use of *A. coynei* to prevent *in vivo* oxidative damage associated with illnesses. Present study also suggests further need for detailed phytochemical investigation and pharmacological studies to support use of this plant by traditional practitioners.

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REFERENCES

1. Lee YM, Kim H, Hong EK, Kang BH, Kim SJ. Water extract of 1:1 mixture of *Phellodendron cortex* and *Aralia cortex* has inhibitory effects on oxidative stress in kidney of diabetic rats. J Ethnopharmacol 2000;73:429-36.
2. Pai SR, Nimbalkar MS, Pawar NV, Patil RP, Dixit GB. Seasonal discrepancy in phenolic content and antioxidant properties from bark of *Nothopodytes nimmoniana* (Grah.) Mabb. Int J Pharm Biosci 2010;1:1-17.
3. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agriindustry by-products: Antioxidant activity, occurrence and potential uses. Food Chem 2006;99:191-203.
4. Shahidi F, Wanasundara PK. Phenolic antioxidants. Cri Rev Food Sci Nutr 1992;32:67-103.
5. Buyukokurouglu ME, Gulcin I, Oktay M, Kufrevioglu OI. *In vitro* antioxidant properties of dantrolene sodium. Pharmacol Res 2001;44:491-4.
6. Pai SR, Upadhya V, Hegde HV, Kholkute SD. *Achyranthes coynei* Santapau, 1949 (Amaranthaceae) – An addition to the flora of Karnataka, India. J Threat Taxa 2011;3:1875-9.
7. *Achyranthes* Linn. (Amaranthaceae). In: Gupta AK, Tandon N, editors. Reviews on Indian Medicinal Plants. Vol. 1. New Delhi: Indian Council of Medical Research; 2004. p. 140-64.
8. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J Agric Food Chem 2003;51:609-14.
9. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Tech 1995;28:25-30.
10. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem 1996;239:70-6.
11. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem 2000;48:4581-9.
12. Sreeramulu D, Raghunath M. Antioxidant activity and phenolic content of roots, tubers and vegetables commonly consumed in India. Food Res Int 2010;43:1017-20.
13. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, *et al*. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem 1998;46:1887-92.

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