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ACHYRANTHES ASPERA LINN: A RESERVOIR OF ANTIOXIDANTS

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ABSTRACT

Antioxidants activities from plants sources have attracted a wide range of interest across the world in recent times. This is due to growing concern for safe and alternative sources of antioxidants. In this study the effectiveness of antioxidant property of *Achyranthes aspera* L. plant was evaluated by quantification of different phytochemicals such as Phenolics, Vitamins, Enzymes from various plant parts at different phases of life cycle. *A. aspera.*, is a good source of Alkaloids, Anthracene Derivative, Cardiac Glycosides Diterpenoids, Phenolic Acids and Polyphenols, Saponines & Sapogenins, Steroids, Sequiterpene Lactones, Triterpenoids and flavonoids. The range for Total Phenolic Content is 0.85-2.88 mg/g , Bound Phenol Content2.33-4.81 mg/g P, Orthodihydroxy Phenol Content 0.06-0.63mg/g ,Tannin content 2.58-8.40mg/g, Flavonols 4.69-13.70mg/g, Quinines 0.002-0.07mg/g, Vitamine A 0.12-0.68 units/mL, Vitamine B3.73-97.96 ug/100g, Vitamine C 83.33-194.44 mg/100g, Ascorbic acid oxidase 0.003-0.005 µ mole ascorbic acid disappeared/min/mg, Catalase 0.1-0.2 units/1min/0.250 gm of sample, Peroxidise 0.002-0.066 units/5 min/0.250gm of sample and Polyphenol oxidase 0.103-1.616 units/3 min/250 mg of plant sample. The results suggest that *A.aspera* is very rich in antioxidant compounds worthy of further investigations.

KEY WORD: ***A.aspera,*** **antioxidant, enzymes, phenolics, phytochemicals, Vitamines**

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INTRODUCTION

Oxidative stress contributes to the development of a wide range of diseases including Alzheimer's disease[1,2]. Parkinson's disease[3] the pathologies caused by Diabetes[4,5], a Rheumatoid Arthritis[6]. Atherosclerosis, Ischemic heart disease, Ageing, Cancer, Immuno-suppression, Neurodegenerative diseases and others[7]. The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is "antioxidative defence" mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties such as enzymatic and non-enzymatic, synthetic antioxidant. The enzymatic antioxidants are produced endogenously which includes superoxide dismutase, catalase, and glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources. There are some synthetic antioxidant compounds such as butylated hydroxyltoluene, butylated hydroxyanisole, tertiary butyl hydroquinone which are commonly used in processed foods[8]. However, it has been suggested that these compounds have shown toxic effects like liver damage mutagenesis. Hence, nowadays search for natural antioxidant source is gaining much importance. Plants based natural constituents can be derived from various parts of plant like bark, leaves, flowers, roots, fruits, seeds, i.e. any part of the plant may contain active components. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potential[9]. With this background and abundant source of unique active components harbored in plants, the present study was taken up on medicinal plant namely *Achyranthes aspera* which is a *common* weed as well as indigenous medicinal plant of Asia, South America and Africa, belonging to the family Amaranthaceae. *A. aspera* is one of the major ingredients in Ayurvedic Preparations[10] reputed to be a laxative, stomachic, depurative, pectoral and astringent*;* its juice is administered in diarrhoea, dysentery, monorrhagia, piles, rheumatism, inflammation of internal organs, coughs*,* enlarged cervical glands, eruptions, boils, *etc. A. aspera* possess abortifacient activity[11-14]*,* hypoglycemic[15], hypolipidemic activity[16], anti-inflammatory activity[17-19], antifungal[20], anti – feedant activity[21]and antibacterial properties[22,23], gynecological disorders[24-28], estrogenic and pregnancy interceptory effects[29], diabetes mellitus[30]. Although many researchers have reported the effectiveness of antioxidant property of the plant by doing DPPH Radical Scavenging Activity[31-34], Hydroxyl Radical Scavenging assay [32,35] reducing power assay[34-36], quantification of different phytochemicals such as total phenols, flavonoids, and tannins[34] . In the present, antioxidant potential of the extracts was determined on the basis of phytochemical screening, and quantification of various phytochemicals such as phenols, enzymes, vitamins by studying different plant parts at different phases of life cycle.

MATERIALS AND METHODS

PLANT MATERIAL

Plants of A. *aspera* was collected fresh in bulk from village Neeri Mankar 25 Km away from Nagpur onn Amaravati road. Specimens collected were identified by Prof. Alka Chaturvedi, Incharge of the herbarium at Department of Botany, RTM Nagpur University, Nagpur where Herbarium specimen with voucher number RTMB 5878 was deposited. The leaves, roots were collected separately from plant dried under shade was then powdered using mechanical grinder.

Phytochemical studies

Preparation of extract

The plant parts of *A. aspera* were coarsely powdered and extracted with different solvents, viz., petroleum ether, ethyl acetate, methanol and water. The extracts were then subjected to preliminary phytochemical

screening as per standard methods prescribed in WHO guidelines[37-38].

Qualitative Phytochemical Screening

The Qualitative Phytochemical screening was done according to the standard procedures adopted for the separation and purification of plant constituents like Alkaloids[39] , Anthracene Derivatives and Anthraqunioines, Cardiac Glycosides, Coumarins and Triterpenoids[40], Diterpenoids[41], phenolic acids[42], polyphenols[43], sapogenins[44], sequiterpene lactones[45], and Steroids[41] were mainly carried out by using chromatographic techniques.

Quantification of Phytochemicals

Total phenols were estimated as per Bray and Thorpe (1954)[46]. Ortho-dihydric phenols, bound –phenols and quinines were estimated as per Mahadevan and Sridhar (1986)[47]. Estimation of Tannins, Vitamine B and Vitamine C were done by the method suggested by Sadasivam and Manickam (1992)[48] .Flavonols and Vitamine A were Estimated as per Thimmaiah (1999)[49].

Estimation of Enzymes

Ascorbic acid oxidase (L-ascorbate:Oxygen oxidoreductase, EC 1.10.3.3), Catalase (EC 1.11.1.6), Peroxidase (E.C 1.11.1.7) and Polyphenol oxidase (E.C 1.14.18.1) were assayed as per Thimmaiah (1999)[49]. Ascorbic acid oxidase activity was assayed by incubating the enzyme with the ascorbic acid substrate. The enzyme oxidizes ascorbic acid and the rate of degradation of it due to oxidation is determined spectrophotometrically. The change in optical density (O.D) value for a unit time is proportional to the activity of the enzyme. Catalase activity was assayed by estimating the residual H2O2 in the reaction mixture which was then determined by oxidation with KMnO4 titrimetrically. The specific activity was expressed as units/min/mg protein. One unit of catalase was defined as that amount of enzyme that breaks down 1µ mole of H2O2 under the assay conditions. Peroxidase activity was assayed using odianisidine as hydrogen donor and H2O2 as electronacceptor. The rate of formation of yellow orange coloured dianisidine dehydrogenation product was a measure of the peroxidase activity and assayed spectrophotometrically at 430 nm. The specific activity of enzyme was expressed as units/min/mg protein considering one unit of enzyme as an increase in optical density (O.D) by 1.0 under standard conditions. Polyphenol oxidase was measured as rate of increase in absorbance colorimetrically at 410 nm with the oxidation of catechol as the substrate. The enzymatic activity was expressed as units min.-1 at 410nm considering one enzyme unit as the change in absorbance of 0.001/minute and the specific activity as units/min/mg protein.

RESULTS

Table No [1] and [2] reveals the rich phytochemical diversity in *A. aspera.* It is a good source of alkaloids, Anthracene Derivative, cardiac glycosides Diterpenoids, Phenolic Acids, polyphenols, Saponines & Sapogenins, steroids, Sequiterpene Lactones, Triterpenoids and flavonoids (Table No [3]). The results of Total Phenol Content, orthodihyroxy phenol and bound phenol are computed and statically analyzed together. Their co-relation between orthodihyroxy phenol content and Total phenol content has been worked out. Table No [4] [5] and [6] .The quantitative determination of ascorbic acid, thamine and carotenoids in plant extracts shows that they are good source . Table no [7]. The results of enzymatic assay reported in table No [8].

Table No 1
Studies in Preliminary Phyotochemical Compounds Results

Chemical compound	Solvent	Leaf	Stem	Root	Fruit
Alkaloids	Acetone	-	+	-	+
	Alcohol	-	+	+	+
	Chloroform	+	+	+	+
	Pt Ether	+	+	-	+
	Water	-	+	-	+
Anthocyanins	Alcohol	+	-	-	+
	Water	+	+	-	-
Anthocyanidins	Alcohol	-	-	-	-
	Water	+	-	-	+
Anthracene Derivative	Alcohol	-	-	+	-
	Water	+	-	-	+
Anthraquinones	Fresh material	-	-	-	-
Cardiac glycosides	70% Alcohol	+	+	-	+
Carotenoids	Pt Ether	+	+	-	+
Coumarins	Acetone,	+	+	-	-
	Alcohol	+	+	-	+
	Chloroform	+	+	+	+
	Pt Ether	+	+	-	+
	Water	-	-	-	-
Cyanogenic glycosides	Fresh material	-	-	-	-
Emodins		-	-	-	-
Fatty acids		+	+	+	+
Flavonoids	Acetone	+	+	-	+
	Alcohol	+	+	+	+
	Chloroform	+	+	+	+
	Pt Ether	-	-	-	-
	Water	-	-	-	-
Iridoids	Fresh material	-	-	-	-
Polyuronoids	Water extract	-	-	-	-
Saponins	Water	+	+	+	+
Steroids	Acetone	-	-	-	-
	Alcohol	-	-	-	-
	Chloroform	-	-	-	-
	Pt Ether	-	-	-	+
	Water	+	+	+	+
Tannins	Alcohol	+	+	+	+
	Water	+	+	+	+
Triterpenoids	Acetone,	+	+	+	+
	Alcohol	+	+	+	+
	Chloroform	-	-	-	-
	Pt Ether	-	-	-	-
	Water	+	+	+	+
Volatile oils	Pt Ether	+	+	+	+

Note: (+) indicates presence and (-) indicates absence

Table No 2
Qualitative Phtyochemical Screening of A. aspera

Chemical Compound	Unknown Compound	Leaf	Stem	Root	Fruit
Alkaloids	U1	0.05	0.1	0.1	0.05
	U2	0.15	0.15	0.15	0.2
	U3	0.25	0.4	0.4	0.3
	U4	0.3	0.55	0.5	0.35
	U5	0.35	0.77	0.55	0.55
	U6	0.72	0.8	0.7	0.6
	U7	0.75	-	0.75	0.65
	U8	-	-	-	0.75
Anthracene Derivative	U1	0.85	-	0.25	0.35
	U2	-	-	-	0.55
	U3	-	-	-	0.75
	U4	-	-	-	0.90
Anthraquinones	U1	-	-	-	-
Cardiac Glycosides	U1	0.72	0.72	-	0.72
	U2	0.80	-	-	-
Coumarins	U1	-	-	0.30	0.17
Diterpenoids	U1	0.06	0.06	-	0.06
	U2	_	0.18	-	0.18
Phenolic Acids	U1	0.33	-	0.56	0.42
	U2	0.36	-	0.58	0.5
Polyphenols	U1	0.07	0.16	0.20	0.21
	U2	0.20	-	-	-
Saponines & Sapogenins	U1	0.14	0.23	0.23	0.25
	U2	0.16	0.26	0.26	0.28
Sequiterpene Lactones	U1	0.38	0.55	0.27	0.41
	U2	0.40	0.58	0.29	0.44
Steroids	*U1*	0.45	0.42	-	-
	U2	0.81	0.78	0.83	-
	U3	-	-	-	-
Triterpenoids	U1	0.62	0.62	0.62	-
	U2	-	0.68	0.68	-
	U3	-	0.81	0.81	-
	U4	-	0.87	0.87	-

Note : U= Represent unknown compound with recorded rf values

Table No 3
Qualitative Phtyochemical Screening of Flavonoids

Mobile phase	Unknown Compound	Leaf	Stem	Root	Fruit
N-butanol: acetic acid: Water (4:1:5)	U1	0.58	0.58	0.66	0.66
Acetic acid: C HCl: Water(10:3:30)	U1	0.91	0.94	0.88	0.88
HoAC	U1	0.82	0.85	0.85	0.85

Note : U= Represent unknown compound

Table No 4
A. aspera Quantitative Analysis of Phenol Content

Chemical Compound	Plant part used/plant life stage	Leaf	Stem	Root	Fruit
Total Phenolic Content mg/g Per gram of dry weight	Vegetative growth	1.18	1.70	1.04	-
	Flowering	2.65	0.85	0.96	-
	Fruiting	1.14	0.74	0.97	2.88
	Senescence	0.92	1.16	0.87	2.71
Bound Phenol Content mg/g Per gram of dry weight	Vegetative growth	4.27	4.05	3.88	-
	Flowering	4.24	4.02	3.48	-
	Fruiting	4.81	2.33	3.10	3.70
	Senescence	4.45	2.33	3.31	3.59
Orthodihydroxy Phenol Content mg/g Per gram of dry weight	Vegetative growth	0.29	0.57	0.16	-
	Flowering	0.63	0.22	0.18	-
	Fruiting	0.26	0.37	0.47	0.50
	Senescence	0.16	0.21	0.06	0.43

Table N0 5
ANOVA Table for Phenol Content

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	Computed F
Total phenol ANOVA cv= 28.96%				
Treatment	15	894.585	17.84	60.067
Error total	32	9.518	0.297	
Bound phenol ANOVA cv=2.83%				
Treatment	15	5.432	0.1306	1.2036
Error total	32	1.959	0.1085	
Orthodihyroxy phenol ANOVA cv = 72.179%				
Treatment	15	16.9189	1.127	4.282
Error total	32	8.739	0.273	

Table No 6
Quantitative Analysis of A. aspera Phenolic Compounds

Chemical Compound	Results in units	Leaf	Stem	Root	Fruit
Tannic cid	mg/g	6.86	2.58	3.76	8.40
Flavonols	Ug/gmatA_{500}	4.69	4.79	5.43	13.70
Quinines	Ug/g	0.002	0.028	0.034	0.07

Table No 7
Quantitative Analysis of A. aspera Vitamins

Chemical Compound	Results in units	Leaf	Stem	Root	Fruit
Vitamin A	USP units per mL	0.38	0.68	0.12	0.45
Vitamin B	Ugthiamine content in 100gsample	68.85	3.73	97.96	56.70
Vitamin C	Inmg/100 g sample	166.66	111.11	83.33	194.44

Table No 8
Enzymes Content in A. aspera plants

Chemical Compound	*Results in units*	Leaf	Stem	Root	Fruit
Ascorbic Acid Oxidase	μ mole ascorbic acid disappeared/min/mg	0.005	0.003	0.004	0.005
Catalase	units/1min/0.250 gm of sample	0.1	0.2	0.2	0.1
Peroxidase	units/5 min/0.250gm of sample	0.05	0.026	0.002	0.066
Polyphenol Oxidase	units/3 min/250 mg of sample	1.616	0.274	0.103	0.45

DISCUSSION

Biomolecules can be oxidized by free radicals which results in oxidative stress. This oxidative damage has an important etiological role in aging and development of diseases like cancer, atherosclerosis, and other inflammatory disorders. Synthetic antioxidants, like butylated hydroxyanisole, are good free radical scavengers; however, the synthetic antioxidants can be carcinogenic. Therefore, there is an increasing interest in searching for antioxidants of natural origin. Several techniques have been used to determine the antioxidant activity *in vitro* in order to allow rapid screening of substances. Various approaches like Estimation of DNA Damage Inhibition Efficiency[50] , DPPH Radical Scavenging Activity [31-34], Hydroxyl radical scavenging assay[32,35], reducing power assay[34-36], and phytochemical analysis[51-52] followed by many workers which reveals high antioxidant potential of *A.aspera.* This study has generated detail account on phytochemical quantification mainly on phenolics. Phenolic compounds are the key phytochemicals with high free radical scavenging activity. It has generated a great interest among the scientists for the development of natural antioxidant compounds from plants. Phenolic compounds also possess anti-mutagenic and anti-tumor activities[53]. Phenolic compounds of plants fall into several categories. Chief among these are the flavonoids which have potent antioxidant activities[54]. Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. Studies on flavonoidic derivatives have shown a wide range of antibacterial, antiviral,anti inflammatory, anticancer, and anti-allergic activities[55-56]. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals[57]. Implicated in several diseases. Flavonoids commonly occur as glycosides in plants. The flavonoid content was mainly affected by different cultivation conditions such as weather conditions, plant location and harvest periods. The content of flavonoids will directly affect the total phenol content of the sample. Genetics also plays a role[58-59]. Phenols and polyphenols exert their protective effects through diverse mechanism such as blocking, interfering or suppressing the activities of enzymes involved in reactive oxygen species generation, quenching free radicals, chelating transition metals to render inactive species[60.] The method of extracting polyphenols from the plant materials is an important factor for determination of antioxidant activity. For example, the recovery of phenols in the extracts of the vegetable matter is associated with the polarity of the solvent[61]. It is known that different phenolic compounds have different responses in the Folin-Ciocalteu method. Similarly the molecular antioxidant response of phenolic compounds varies remarkably, depending on their chemical structure[62]. In addition, there may be some interference rising from other chemical components present in the extract, such as sugars or ascorbic acid[63]. Phenolic compounds i.e. Total phenol content, orthodihyroxy phenols, and bound phenols have received considerable attention owing to their association with passive and active defense responses to disease. Ortho-dihydroxy phenols are important in disease resistance reaction, they are easily oxidized

by phenol oxidases and their resulting quinines are highly reactive and toxic to pathogen and their enzymes. The bound phenols occur in esterified form with cell wall components in plants. Their possible role in disease resistance is well known. Total phenol content and orthodihyroxy phenol content varies significantly with different phase of plant life cycle and are highest at flowering in this plants. The bound phenol did not vary significantly with the different phase of plant life cycle. Tannins are the polyphenolic compounds and are considered as an antinutritional factor as they lower digestibility and reduction of food consummation. Quinines are colored natural pigments widely distributed in higher plants. Quinines consist of four sub groups as benzoquinones, napthaquinones, and anthraquinones and isoprenoids quinines. Their studies are conducted mainly because certain anthraquinones have a catharatic action. Flavonols occur in higher plants in addition to the antocyanins. There are over hunders of types Flavonols known. Saponins are natural products, which have been shown to possess antioxidant properties[64,65]. It was proved that carotenoids have a positive role on the epithelisation process and influence the cell cycle progression of the fibroblasts[66]. Carotenoids act as photoprotective agents and may reduce the risk of sunburns, photo-allergy and even some types of skin cancer[67]. The biologically active form of thiamine is thiamine pyrophosphate (TPP), which serves as the coenzymes for oxidative decarboxylation of ∝-keto acids in carbohydrate metabolism. Ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine[68,69] . Different enzymes have different mode of action in imparting resistance. Increased peroxidase activity has often been studied in connection with the oxidation of phenolic substances in the diseased plants and resistance in host attributed to toxicity of these oxidation products[70]. Moreover, enhanced peroxidase activity has been linked with synthesis of lignin[71&72]. Catalase activity increased during infection as a mechanism to scavange fungitoxic H_2O_2[73]. Polyphenol oxidase is commonly found in plants. It oxidizes phenols to highly fungitoxic quinones and is highly inhibitory to some plant fungi[74]. It has been observed that *Parthenium hysterophorus* infected with *X.axonopodis* shows increased levels of peroxidase and catalase[75]. There are reports on cytotoxicity against cultured human hepatoma cells (SMMC-7721) and human acute promyelocytic leukemia cells (HL60) due to presence of Triterpenoids and Sesquiterpenes in *Mulgedium tataricum*[76]. The inhibitory action of triterpenoidal saponin from *Albizia julibrissin* against Bel-7402 cancer cell line[77]. Reports revals that *Solanum nigrum* extract, cytotoxicity with IC50 was due to presence of steroidal saponins, solanigrosides C-H (2-7), and one known saponin, degalactotigonin[78].

CONCLUSION

Thus the carried out analysis proves that *A. aspera* is good reservoir of the antioxidants. The references about this compunds suggest that it is promising taxa for remedy against cancer, cardiovascular disease, Alzheimer's disease. Currently we are not having that much effective remedy against these disorders. It is needed to carry out further studies in details to test the effectiveness of these plants against these burning problems. Also this plant is a promising anti-allergic, anti-inflammatory, anti-microbial and anti-Malarial agent. This research also suggests that *A. aspera* can be a proficient alternative medicine for infectious diseases encountered today which could raise the optimism of scientists about the future of phytomedicine. In future, this study can be further extended *in vivo* in animal cell lines and tissue cultures in the aim of discovering a new drug to combat human diseases.

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