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Phenolic contents and antioxidant activity of *Aloe* strains grown in middle hill climatic conditions of western Himalayas

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ABSTRACT

The present study was carried out to evaluate the antioxidant activities, total phenolics, tannins and ascorbic acid content in dried powder of 8 Aloe vera strains collected from different area of India. The antioxidant property was evaluated by measuring scavenging effect of 2, 2-diphenyl-1-picryhydrazyl (DPPH) radical. The total phenolics, tannins and ascorbic acid content in extracts of different strains were also determined to correlate with their differential antioxidant activity. Whole leaf powder of different strains exhibited better antioxidant activity as compare to gel powder. The maximum antioxidant activity (73.88 %) was recorded in whole leaf powder of strain *collected form Pithoragarh Ghati area. While maximum phenolics (0.70 %) and tannins (0.78 %) were found in dried powder of* Aloe vera *strain collected from Haldwani area. Higher ascorbic acid content (3.652mg/100g) was found in whole leaf powder of A. vera strain collected form Pithoragarh Ghati area. The antioxidant activity in strains is mainly because of the presence of α-tocopherol (vit. E), carotenoids, ascorbic acid, flavonoids etc rather than phenolics and tannins contents.*

INTRODUCTION

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc [4,11]. Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders [15]. Almost all organisms are protected up to some extent by free radical (peroxide, hydro-peroxide or lipid peroxyl) damage with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds viz. ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids and glutathione [13]. However, antioxidant supplements or dietary antioxidants may be source of protection that the body needs to protect against the damaging effects of free radicals [1, 13]. Presently, much attention has been focused on the use of natural antioxidants

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to protect the human body especially brain tissues from the oxidative damage caused by free radicals. In last two decades, several medicinal plants have shown such effectiveness through the traditional methods of psychoneuropharmacology [2].

Aloe vera is a perennial plant belonging to the family of Liliaceae [8]. *Aloe* is the one of the few medicinal plants that has maintained its popularity from ancient time. *Aloe vera* contains substantial amounts of antioxidants including á-tocopherol (vitamin E), carotenoids, ascorbic acid (vitamin C), flavonoids and tannins [9]. Currently, the plant is widely used in skin care, cosmetics and as nutraceuticals [3, 14]. In view of the above, the study was conducted to evaluate the antioxidant properties of methanolic extract of different strains of *Aloe vera* in middle hill climatic conditions of western Himalyas. The total contents of the phenolics, tannins and ascorbic acid present in different strains of *Aloe vera* were also determined to correlate with their antioxidant activity.

MATERIALS AND METHODS

Plant Material

Eight strains of A*loe vera* were collected from different area of the India list Pithoragarh ghati, Lambgara (Nainital), Champawat, Raipur (MP), Dehradun, Haldwani, Baste (Pithoragarh) and Betalghat (Nainital) and coded as DARL-1 to DARL-8 and are being multiplied in open and protected condition in this institute. The mature leaves of all the 8 strains were collected and dehydrated in dehydration chamber below 40 ^{o}C and then powdered with a mechanical grinder and stored in an air-tight container. The dried powder materials of the plants were defatted with petroleum ether and the marc thus obtained was then extracted with methanol in a Soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained. The plants materials were dried by lyophilizer and were used for evaluation of DPPH free radical scavenging activity, total phenolics, total tannins and ascorbic acid content in the present study.

Chemicals

Caffeic acid, indigo solution, 1,1-diphenyl-2-picryl - hydrazyl (DPPH), ascorbic acid, tannic acid and oxalic acid were purchased from Sigma Chemical Co. Ltd, USA. Sodium carbonate, $KMnO_4$, 2,6-dichlorophenolindophenol, and other chemicals of analytical grade were purchased from E. Merck and were used without further purification.

Determination of antioxidant activity

DPPH free radical scavenging activity (FRSA) has been used by various researchers as a quick and reliable parameter to assess the in vitro antioxidant activity of crude plant extracts [12, 19]. The method for estimating FRSA of the methanolic extracts was undertaken as suggested by Hatano *et al.* [5]. The DPPH reagent evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants [1]. Two ml of methanolic solution of DPPH (0.1 mM) was mixed with 200 µl of extract (0.2 mg/ml) at various concentrations in methanol and final volume of 3 ml was made. The absorbance of the mixture was measured after 40 minutes at 517 nm against methanol as blank. Ascorbic acid was used as standards. The FRSA (%) of tested samples were evaluated by comparing with a control (2 ml DPPH and 1 ml of methanol). Each sample was then measured in triplicate and averaged. The FRSA was calculated using the formula: FRSA = [(Ac-At)/Ac-As]x 100, Where Ac = Absorbance of the control, As = Absorbance of the standard and At = absorbance of the tested sample after 40 min.

Determination of total phenolic content

The concentration of total phenols in the extracts was determined by the using Folin-Ciocalteu method [10] and external calibration with caffeic acid (Sigma, St Louis USA). Extract solution (0.2 ml) and Folin-Ciocalteu reagent (0.2 ml) were added and the contents mixed thoroughly. After four minutes, 15% sodium carbonate (1 ml) was added, and the mixture was allowed to stand for two hours at room temperature. The absorbance was measured at 760 nm. The concentration of

the total phenolics was measured as percentage of caffeic acid equivalent by using an equation obtained from the caffeic acid calibration curve.

Determination of total tannin content

Total tannin content in different *Aloe vera* strains was determined by using Folin–Denis Method [17]. Two gram of powdered drug was extracted for 20 h with petroleum ether. The residue was boiled for 2hr with 300 ml of double distilled water. It was cooled, filtered with Whatman filter paper and diluted to 500ml with double distilled water. 25 ml of this infusion was pipetted into 2 litre porcelain dish to which 20 ml indigo solution and 750 ml double distilled water was added. This was titrated with standard $KMnO_4$ (0.1 N) solution by adding 1 ml at a time, until blue solution changed to green, after which a few drops were added at a time until solution turned green yellow in colour (a). Similarly, a mixture of 20 ml indigo solution and 750 ml of double distilled water was titrated (b). The percentage of total tannins was calculated using the formula, % Total tannins = [(a-b) × Actual Normality of $KMnO_4$ solution × 0.004157 × 1000] / Weight of drug sample taken × 0.1 each ml of 0.1 N $KMnO_4$ = 0.004157 g of total tannins.

Determination of ascorbic acid content

Total ascorbic acid content in plant extract was determined by 2, 6- dichlorophenolindophenol method [16]. Two g dried powder of whole leaf was extracted with 4% oxalic acid and the volume was made up to 100 ml. It was centrifuged at 10,000 rpm for 10min. 5 ml supernatant liquid was transferred to a conical flask and 10 ml of 4% oxalic acid was added. It was titrated against standard dye solution (2, 6-dichlorophenolindophenol) to a pink end point. The procedure was repeated with a blank solution (without adding sample). 5 ml ascorbic acid of 100 ppm was used as standard. Ascorbic acid content was calculated using the formula: Ascorbic acid (mg/100 g) =[0.5mg × titer vol against test × 100 ml/titer vol. against ref. × 5 ml × weight of sample] × 100.

Statistical analysis

Statistical analysis of difference between two strains was done by one-way ANOVA followed by Student's t test. $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

The antioxidant properties of *Aloe vera* strains have been evaluated by measuring their DPPH free radical scavenging activity, total phenols, total tannins and ascorbic acid contents using extracts of whole leaf powder and leaf's gel powder of the plant.

Antioxidant activity using DPPH assay

The results of FRSA of methanolic extracts of different strains of *Aloe vera* are shown in table 1. It is evident from results that FRSA of whole leaf powder of *Aloe vera* strains varied from 42.79% to 73.88 % in protected cultivation condition and 59.32 to 86.32 % in open cultivation condition. Maximum FRSA (73.88%) in protected cultivation and (86.32 %) in open cultivation condition were found in *Aloe vera* strain DARL-1 collected from Pithoragarh Ghati area. While, least FRSA (42.79%) in protected cultivation and (59.32 %) in open cultivation condition were observed from DARL-7 (Baste, Pithoragarh). While, in gel powder, FRSA was varied from 18.75 to 53.86% in protected cultivation condition and 27.62 to 64.97 % in open cultivation condition. Highest FRSA (53.68%) in protected cultivation and (64.97%) in open cultivation condition was recorded from gel of DARL-7 (Baste, Pithoragarh). Whereas, Ascorbic acid at the 2.5 µg/ml concentration exhibits 100% FRSA. In the present study significantly higher FRSA was found in open cultivation condition compared to protected cultivation condition. Thus study concluded that very good antioxidant activity was found in *Aloe vera* strains when grown in open condition. Whole leaf powder of *Aloe vera* exhibited better antioxidant activity compare to gel powder.

Table 1: DPPH free radical scavenging activity (FRSA) of different strains of *Aloe vera* in open and protect condition

S. No.	*Aloe Vera* Strains	FRSA (%) in Whole Leaf Powder		FRSA (%) in Gel Powder	
		Protected Condition	Open Condition	Protected Condition	Open Condition
1	DARL-1 (Pithoragarh Ghati)	73.88	86.32	19.41	27.62
2	DARL-2 (Lamgara)	64.55	78.45	32.68	40.75
3	DARL-3 (Champawat)	58.84	75.62	28.68	35.64
4	DARL-4 (Raipur)	64.35	80.06	32.19	49.52
5	DARL-5 (Dehradun)	63.62	79.65	44.50	57.84
6	DARL-6 (Haldwani)	63.12	76.98	37.67	44.61
7	DARL-7 (Baste, Pithoragarh)	42.79	59.32	53.68	64.97
8	DARL-8 (Betalghat)	54.89	64.85	18.75	25.46

Total phenolic contents

Phenols are another important plant constituent due to their free radical scavenging ability because of hydroxyl groups [6]. Tanaka et al. [18] had suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when consumed up to 1g per day through diet rich in fruits and vegetables. The total phenolics contents of different *Aloe vera* strains range from 0.35 to 0.70% in dried leaf powder. DARL-6 collected from Haldwani and cultivated in open condition contained the highest total phenolics contents (0.70%), followed by DARL-4 (0.52%) collected from Raipur and cultivated in open condition. Results of total phenolics of the dried powder of *Aloe vera* strains are illustrated in Table 2.

Total tannin contents

Tannins also possess very high antioxidant activity due to their tremendous free radical scavenging ability and thus they protect the body from harmful effect of free radicals. It is evident from the data that total tannin content of the dried powder of *Aloe vera* varied from 0.42% to 0.78%. Sample of *Aloe* strains DARL-6 (Haldwani) cultivated in open condition contained the highest total tannin contents (0.78%), followed by *Aloe* strain DARL-4 (0.56%) collected from Raipur and cultivated in open condition. The result of total tannin contents of the dried powder of *Aloe vera* from different locations was illustrated in Table 2. This result was similar to the studies of Zhou & Yu [20], where they evaluate the bran extracts from various localities in Colorado, USA and Khamsah *et al*. [7] has been revealed that extract of *O. stamineus* benth from different geographical origin in Malaysia.

Ascorbic acid contents

Ascorbic acid is a naturally occurring antioxidant compound found in medicinal plants, vegetables, fruits and whole grains. The content of ascorbic acid in different *Aloe vera* strains was recorded from 1.681 to 3.652mg/100g in dried powder of whole leaf. We observed that DARL-1 *Aloe vera* strain collected from Pithoragarh Ghati area and cultivated in open condition have higher ascorbic acid contents (3.652 mg/100g) followed by DARL-4 (2.425 mg/100g) collected from Raipur and cultivated in open condition as compared to other *Aloe vera* strains. Data of ascorbic acid content of the dried powder of *Aloe vera* strains are illustrated in Table 2.

CONCLUSSION

It is evident from the present study that the methanolic extract of whole leaf powder of different strains of *Aloe vera* exhibited better antioxidant activity as compare to gel powder. The whole leaf powder of *Aloe vera* strain DARL-1 collected from Pithoragarh Ghati area and cultivated in open condition has best antioxidant activity followed by

Table 2: Total phenolics, tannin and ascorbic acid contents in different strains of *Aloe vera* cultivated in open condition.

S. No.	*Aloe Vera* Strains and Collection Places	Total Phenolic content (%)	Total tannin content (%)	Ascorbic acid mg/100gm
1	DARL-1 (Pithoragarh Ghati)	0.32	0.42	3.652*
2	DARL-2 (Lamgara)	0.33	0.46	2.057
3	DARL-3 (Champawat)	0.35	0.44	2.144
4	DARL-4 (Raipur)	0.52	0.56	2.425
5	DARL-5 (Dehradun)	0.40	0.45	2.374
6	DARL-6 (Haldwani)	0.70*	0.78*	1.768
7	DARL-7 (Baste, Pithoragarh)	0.37	0.53	1.847
8	DARL-8 (Betalghat)	0.49	0.46	1.681

strain DARL-2 (Lamgara). While in case of gel powder, maximum antioxidant activity was found in A*loe* strain DARL-7 collected from Baste, Pithoragarh and cultivated in open condtion followed by DARL-5 (Dehradun). Total phenolics and tannins contents were found highest in DARL-6 *Aloe vera* strain (Haldwani, Nainital) region cultivated in open area followed by DARL-4 (Raipur) cultivated in open area. The maximum ascorbic acid content was found in DARL-1 which is collected from Pithoragarh Ghati area and cultivated in open condition. Hence, antioxidant activity in the *Aloe vera* strains is mainly because of the presence of antioxidant compounds like á-Tocopherol (Vit. E), Carotenoids, ascorbic acid, flavonoids etc rather than phenolics and tannins contents.

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