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Impact of spatial and climatic conditions on phytochemical diversity and in vitro antioxidant activity of Indian *Aloe vera* (L.) Burm.f.

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ABSTRACT

The aim of present study was to focus on the impact of spatial and different climatic conditions on phytochemical diversity and antioxidant potential of aqueous leaf extracts of *Aloe vera* collected from different climatic zones of India. Crude aqueous extracts of *Aloe vera* from different states varied in climatic conditions of India were screened for phytochemical diversity analysis and in vitro antioxidant activity. Phytochemical analysis was performed with the help of Fourier Transform Infrared (FTIR) Spectroscopy. DPPH free radical scavenging assay, metal chelating assay, hydrogen peroxide scavenging assay, reducing power assay and β carotene-linoleic assay were used to assess the antioxidant potential of *Aloe vera* aqueous leaf extracts. FTIR analysis in present study showed presence of various phytoconstituents from different *Aloe vera* samples. All antioxidant assays revealed that Highland and Semi-arid zone samples possessed higher antioxidant activity whereas Tropical zone samples possessed minimum. It could be concluded that different agro-climatic conditions have effects on phytochemical diversity and antioxidant potential of *Aloe vera* plant. This study demonstrated that antioxidant activity was higher in *Aloe vera* plants grown in Northern India in comparison to Southern India. Study also concluded that more phytochemicals are produced in plants under cold stress conditions. *Aloe vera* can be a potential source of novel natural antioxidant compounds.

1. Introduction

India is located between 8°-30° N and 68°-97.5° E. There is an extreme variation in altitude-from sea level to heights upto Himalayas. India is floristically rich having about 33% of botanical wealth is endemic. Indian Council of Agriculture Research recognized 8 agro-climatic regions on the basis of physiographic, climatic and cultural features. Different agro-climatic/phyto-geographical regions of India hold rich diversity in both wild and cultivated plant gene pools. There are about 1500 medicinal plants in India. Traditional societies still use native wild plants for medicinal purposes (Paroda and Arora, 1991). *Aloe vera* grows in arid climates and it is widely distributed in India.

Aloe vera (L.) Burm. f. (Synonym *A. barbadensis* Miller) is a succulent perennial plant with green, tapering, spiny, marginated and dagger-shaped fleshy leaves filled with a clear viscous gel (Klein and Penneys, 1988). *Aloe vera* is the most commercialized *Aloe* species belonging to the Xanthorrhoeaceae family. This plant grows readily in hot and dry climates (Vogler and Ernst, 1999). *Aloe vera* has its origin on the Arabian Peninsula (Grace et al., 2015) although it is also known in the Mediterranean, the American subcontinents, and India. *Aloe vera* juice has been used traditionally for its purgative effects and fresh leaf gel used in different formulations and cosmetic preparations (Wani et al., 2010). *Aloe vera* contains over hundreds of nutrient and bio-active compounds, including vitamins, enzymes, minerals, sugars, lignin, anthraquinones, saponins, salicylic acid and amino acids, which are responsible for their medicinal properties (Park and Jo, 2006). Its secondary metabolites have multiple properties such as anti-inflammatory (Bhattacharjee et al., 2014), antibacterial (Kumar et al., 2016), antioxidant (Nejatzadeh-Barandozi, 2013), immune boosting, anticancer, antidiabetic, anti-ageing and sunburn relief (Langmead et al., 2004; Rishi et al., 2008; Wintola and Afolayan, 2011). Several uses of *Aloe vera* also have been reported such as for burn injury, eczema, cosmetics, inflammation and fever in traditional medicine systems (Jayaprakasha et al., 2001).

Reactive Oxygen Species (ROS) is a term used to describe reactive oxygen and nitrogen species that are common outcome of normal aerobic cellular metabolic processes. During daily activities and with advanced age, oxidative substances and free radicals accumulate in cells affecting various organs and systems in our body (Uttara et al., 2009; Lobo et al., 2010). Overproductions of these free radicals are the results of chronic and other degenerative diseases in humans (Freidovich, 1999; Fang et al., 2002). Uncontrolled production of these free radicals leads to attack on various biomolecules, cellular machinery, cell membrane, lipids, proteins, enzymes and DNA causing oxidative stress and

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ultimately cell death (Tiwari, 2004). Defense against free radicals can be enhanced by taking sufficient amounts of exogenous antioxidants (Yadav et al., 2014). Micronutrients like vitamin E, β carotene, and vitamin C are the major antioxidants. These must be provided in diet as body cannot produce these nutrients (Ramassamy, 2006).

Medicinal plants are the richest bioresource of drugs for traditional systems of medicine. Since evolution, man has been using plant extracts to improve his health and life-style. Prime sources of naturally occurring antioxidants for humans are fruits, vegetables and spices. Search for the novel natural antioxidants from tea, fruits, vegetables, herbs, and spices are continued as efforts have been made by researchers all over the globe. Herbs are on focus of whole world as a source of novel antioxidant compounds due to their safety as compared to synthetic antioxidants. Many plants have been screened for their antioxidant potential and there is growing interest in replacing synthetic antioxidants because of the concern over the possible carcinogenic effects of these in foods with natural ingredients (Shahidi and Zhong, 2010). Medicinal plants contain a wide variety of free radical scavenging molecules such as phenolic compounds (Phenolic acids, flavonoids, catechins, proanthocyanidins, quinons, coumarins, tannins etc.), nitrogen compounds (alkaloids, amines, betalains etc.), vitamins, terpenoids, carotenoids and other secondary metabolites which are reported to have antioxidant activity (Cotelle et al., 1996; Velioglu et al., 1998; Zheng and Wang, 2001; Cai et al., 2003). Several techniques have been used to estimate and determine the presence of such bio-active phytoconstituents in medicinal plants. Chromatography and spectroscopic techniques are the most useful and popular tools used for this purpose. Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is a characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined (Ashokkumar and Ramaswamy, 2014). FTIR spectroscopy allows the analysis of a relevant amount of compositional and structural information in plants. Moreover, it is an established time-saving method to characterize and identify functional groups (Grube et al., 2008).

The changing temperatures and wind patterns associated with climate change are causing noticeable effects on the life cycle, distribution and phyto-chemical composition of the world's vegetation, including medicinal and aromatic plants (Aryal, 2015). Alongside its diverse geography, India is a home to extraordinary variety of climatic conditions; ranging from tropical in the south to temperate and alpine in the Himalayan north, where elevated regions receive sustained winter snowfall. These vast climatic variations may cause a difference in the phytoconstituents of plants. Previous studies state that phytochemical composition of plants is influenced by a variety of environmental factors including the geography, climate, soil type, sun exposure, grazing stress, seasonal changes etc. (Ganskopp and Bohnert, 2003; Khan et al., 2006; Hussain et al., 2009). Present study focuses on to predict effects of geographical and climatic conditions on phytochemical diversity of *Aloe vera* from all the 6 different agro-climatic zones of India. Crude aqueous leaf extracts of different *Aloe vera* samples were also used to evaluate antioxidant potential of the plant.

2. Material and methods

2.1. Collection of plant material

Samples were collected from 12 different sites covering 6 agro-climatic zones of India. Each zone had 2 sites (Fig. 1). Geographical locations of collection sites along with their average temperature and rainfall are depicted in Table 1. Samples were collected in the months of Jan–Feb 2013 from naturally growing plants. Healthy leaves of *Aloe vera* were collected from five individual plants from each location and placed in sterile plastic bags. All samples were brought to the laboratory in an ice box and processed further. Fresh gel of the plant is used for skin treatments (facial and cosmetic etc.) and dry form of the plant used locally in folk medicine as a laxative. The plant material was identified and authenticated by comparing the herbarium specimen (MDU-6803) available in Department of Genetics, M. D. University, Rohtak (India) and voucher specimens were deposited in departmental herbarium. Sample collection pictures from different climatic zones are depicted in Plate 1.

2.2. Preparation of extracts

Leaves were washed with running tap water and dried in shade. Dried leaves were crushed using a willey mill. Aqueous extracts of different samples were prepared by cold percolation method. To 100 g of dried leaf mass 1 L water was added in a flask (1:10). It was incubated in an incubator shaker at 28°C for 48–72 h. at 180 rpm. Extract was filtered through Whatman filter paper No. 1. Filtrate was concentrated with help of rotary vacuum evaporator at 40°C. Dried mass was weighed. Table 2 shows the yield of extracts from different samples.

2.3. Phytochemical screening of Aloe vera extracts

The crude aqueous *Aloe vera* extracts were characterized using a Fourier transform infrared spectrophotometer (FTIR 8400S, Shimadzu, Tokyo, Japan). 2 mg of the sample was mixed with 100 mg potassium bromide (KBr). Then, compressed to prepare a salt disc approximately 3 mm in diameter and the disc were immediately kept in the sample holder. FTIR spectra were recorded in the absorption range between 400 and 4000 cm^{-1}.

2.4. Antioxidant assays

Five generally used methods as; DPPH free radical scavenging assay, Hydrogen peroxide scavenging assay, Reducing power assay, metal chelating assay and β carotene-linoleic assay were used to assess the antioxidant potential of *Aloe vera* aqueous leaf extracts. Each experiment was done in triplicates and mean values were interpreted to conclude the results.

2.4.1. DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity

Antioxidant activity of crude aqueous *Aloe vera* extracts were measured in terms of hydrogen donating radical scavenging ability using the stable DPPH method of Zhu et al. (2006). The reaction was monitored as a color change from purple to pale yellow. One milliliter (1 mg/ml) of *Aloe vera* extracts were added to 2 ml of 0.5 mM DPPH solution in methanol. Reaction mixture was kept in dark for 45 min. Absorbance was recorded at 517 nm against blank using a UV–Vis Spectrophotometer. Ascorbic acid was used as a standard. The radical scavenging activity on DPPH was expressed as,

$$\text{Scavenging effect}(\%) = [(A_o - A_1)/A_o] \times 100$$

where A_o is the absorbance of control and A_1 is the absorbance of sample extract or standard.

2.4.2. Hydrogen peroxide scavenging (H_2O_2) assay

Extracts ability to scavenge hydrogen peroxide was estimated by following the method of Ruch et al. (1989). The extracts were dissolved in phosphate buffer at a concentration of 1 mg/ml. 1 ml of extract was added to 3.4 ml of phosphate buffer (50 mM, pH 7.4). 600 μl of 400 mM H_2O_2 was added to the solution. The solution was kept at room temperature for 40 min. The absorption was measured at 230 nm. Ascorbic acid was used as control.