Research Article

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EFFECTS OF SEASONAL VARIATION ON ANTIOXIDANT POTENTIAL AND TOTAL PHENOLIC CONTENT OF *ZANTHOXYLUM ARMATUM*

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ABSTRACT

The Study evaluates effects of Seasonal Variation on Antioxidant Potential and Total Phenolic Content of *Zanthoxylum* armatum. DPPH and FRAP methods were used to evaluate effect of seasonal discrepancy on the antioxidant potential of plant *Zanthoxylum armatum*. The investigation observed that the antioxidant activity depends upon total phenolic and flavonoid contents which may affect by seasonal discrepancy. Study observed abundant of phenolic and flavonoid during summer season resulting potent antioxidant activities. The moderate antioxidant activity was observed in monsoon season. Study concluded that total phenolic content increased from rainy to summer season and thus antioxidant activities also vary with seasonal discrepancy.

Keywords: *Zanthoxylum armatum,* antioxidant potential, seasonal discrepancy, phenolic content, DPPH and FRAP methods.

INTRODUCTION

Zanthoxylum armatum belongs to family *Rutaceae* is a very common plant of Asia, it occurs hilly area of India and Pakistan. The aerial parts of plant used as stomachic and carminative, also used for toothache. The plant seeds and bark are used in fevers, heartburn and in cholera[1-3]. It also possesses antimicrobial, larvicidal, abortifacient, antifertility, antiseptic, anti-diarrheal and cytotoxic activities. The various phytoconstituents present in plants; including terpenes, sterols, flavonoids, alkaloids and coumarins[3-5].

Polyphenols are the aromatic hydroxylated compounds present in plant and possess significant therapeutic value. The phenolic compounds have been studied extensively since they are present in most of the plants and considered as biologically active constituent. These polyphenols possessed radical scavenging ability, anticancer and antiviral activities. The literature survey revealed that thousands of phenolic compounds obtained from various natural sources. Polyphenols obtained from plant act as natural hydrogen donor and reducing agents. The ability to transfer hydrogen atom, electron donation, and metal chelation, interaction with other antioxidants, localization and mobility of the antioxidant decides antioxidant potential of polyphenols obtained from natural sources[6-9].

Recently many researches paid great attention towards the seasonal variation in plant secondary metabolites and it has been established that the photochemical content of plant. There are various factors which affect production of secondary metabolites such as; light, temperature, soil and seasonal variation etc. The seasonal discrepancy play significant role towards the production of polyphenols thus biological profile of plants which are rich in polyphenols contents also changes along with seasons[8-10]. The present work describes the effects of seasonal variation on antioxidant potential and total phenolic content of *Zanthoxylum armatum.*

MATERIAL & METHODS

Sampling

The collection of plant samples was done in the month of Aug, Dec and May; year 2007-08. These months were selected since they represent monsoon, winter and summer seasons, respectively. The collected samples were dried and powdered for further use.

Extraction

Powdered plant materials of *Zanthoxylum armatum* were extracted with methanol[11]. The extracts were then cooled and filtered through filter paper (Whatman No. 1) followed by centrifugation for 10 min, diluted 1:15 with same solvent and used for further experiments except for HPLC analysis, where the extract was injected directly without dilution after filtering it through a 0.2 µ nylon filter.

Determination of total phenolic content

Total phenolic content was determined using modified Folin – Ciocalteu method[12]. The extract was diluted with distilled water to a known concentration in order to obtain the readings within the standard curve range of 0.0 to 600 µg of tannic acid/ml. 250 µl of diluted extract or tannic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 µl of Folin - Ciocalteu reagent. The sample was mixed well and then allowed to for 5 min at room temperature in order to allow complete reaction with the Folin-Ciocalteu reagent, that after 2.5 ml of 7 % sodium carbonate aqueous solution was added and the final volume was made up to 6 ml with distilled water. The absorbance of the resulting blue colour solution was measured at 760 nm using spectrophotometer after incubating the sample for 90 min.

Antioxidant Activity
DPPH Radical Scavenging Assay

The antioxidant property of plant was measured using DPPH radical scavenging method. The antioxidant activity was compared with ascorbic acid. The antioxidant activity of plant extracts collected in various seasons was expressed in terms of IC_{50}[13]. 1.5 ml of 0.1 mM DPPH solution was mixed with 1.5 ml of various concentrations of extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solution without any extract along with DPPH and methanol was used as control. Inhibition of DPPH free radical in percentage was calculated by the formula:

$$\text{Inhibition (\%)} = [(A_{control}-A_{test})/A_{control}] \times 100$$

Where; $A_{control}$ is the absorbance of the control (L-Ascorbic acid) A_{test} is the absorbance of reaction mixture sample (in the presence of sample).

Determination of Reducing Property

The reducing power of the plant extract was determined by a slightly modified method[14]. 1 ml of each plant extract concentration was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1 %). The mixtures were then incubated at 50°C for 20 min. Aliquots (2.5 ml) of trichloroacetic acid (10 %) was added, then centrifuged for 10 min. The upper layer of the solutions (2.5 ml) was mixed separately with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %), the absorbance was measured at 700 nm using a spectrophotometer. Solvent without extract was used as control. BHT (Butylated Hydroxy Toluene) was used as standard.

HPLC Analysis

The HPLC apparatus equipped with multi solvent delivery system and UV dual detector. Chromatographic separation was achieved on a C_{18} column, methanol: ethyl acetate: glacial acetic acid (85:14:01) used as mobile phase in a linear gradient programme, the flow rate of the mobile phase was maintained at 1.0 ml/min, peaks were detected at 280 and 360 nm. The stock solutions of standards were diluted serially with solvent to prepare calibration curves at different concentration levels. The concentrations were determined using calibration curves and peak areas. The average content was calculated as a mean of 3 different readings obtained at different seasons[15].

RESULTS AND DISCUSSIONS
Determination of total phenolic content

The total phenolic content of *Zanthoxylum armatum* samples collected in different seasons was determined and depicted in Table 1. The total phenolic content ranged from 4.92 ±0.27 to 22.32 ±3.13 g/100g TAE. It was high in sample extracts collected during summer season, while low content was observed for the samples collected during monsoon season. A pattern of increase in phenolic content was observed from season of monsoon < winter < summer. The result suggested that the climatic conditions including temperature act as regulating factor to produce total phenolic content in plant. The seasonal variation affects rate and extent of various biochemical processes which are responsible for the production of secondary metabolite and therefore the phenolic content of plant varies along with seasons.

Table 1: Total phenolic content of sample of *Zanthoxylum armatum* collected during different seasons

Month of Collection	Total Phenolic Content (g/100g TAE)
August	4.92 ±0.27
December	12.14 ±0.21
May	22.32 ±3.13

Antioxidant Activity

Free radical scavenging potentials of plant *Zanthoxylum armatum* determined using DPPH and BHT reducing power assay presented in Figure 1. The results were expressed as Ascorbic acid equivalent antioxidant capacity (AEAC) and Trolox equivalent antioxidant capacity (TEAC). Samples collected during summer season showed the most potent radical scavenging activity than samples collected in other months. The moderate and lowest activity was observed in winter and rainy season respectively. The antioxidant activity in DPPH assay ranging from 261.01 ±11.30 to 650.66±06.35 µM AEAC increases from rainy to summer season while antioxidant activity in BHT reducing power assay ranged from 191.75 ±13.25 to 417.52 ±20.51 µM TEAC. The pattern of the antioxidant activity observed for samples collected in different seasons is similar to the patterns observed in total phenolic content of plant; means antioxidant potential increases from monsoon to summer season. This variation attributed to the fact that low temperature and high rainfall significantly reduces production of phenolic content.

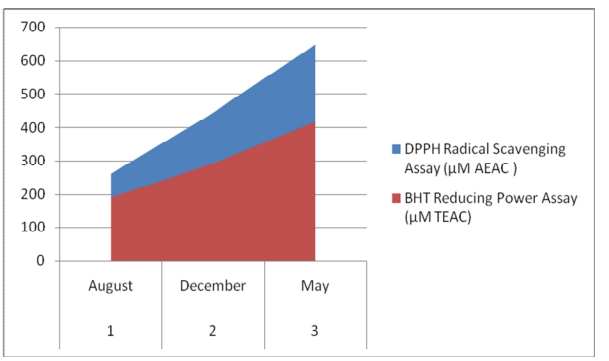

Figure 1: Increasing antioxidant potential of plant samples collected from month of August < December < May.

HPLC Analysis

The HPLC analysis represents phenolic profile of each sample in ppm equivalent to tannic acid (Figure 2). Calibration curves for standard were found to be linear with $R^2 = 0.999$. The HPLC profiles showed several peaks since natural compounds consisted of many constituents. The methanolic extract of sample collected in the month of May showed highest tannic acid content (389.23 ppm) and lowest in the sample collected during month of Aug (212.31 ppm). HPLC analysis yielded phenolic profiles in various retention times. The HPLC analysis observed different other peaks also along with phenolic compound between RT 0 – 20 min. Phenolic compound separated with good resolution. The finding of HPLC analysis resembles that the potent antioxidant activity of plant sample collected in the month of May due the high amount of phenolic compound. The results of HPLC analysis suggested that the amount of phenolic compound in plant *Zanthoxylum armatum* vary as per seasonal variation and this variation affect antioxidant potential of plant.

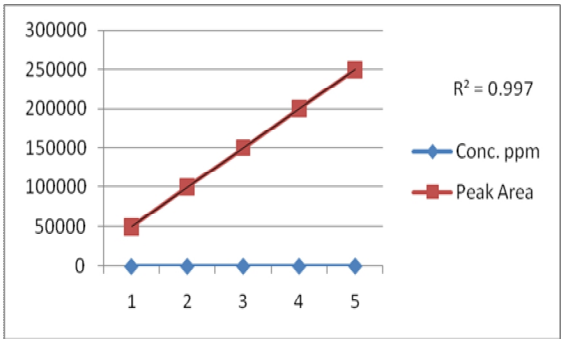

Figure 2: Calibration graph of phenolic compound showed linear relationship

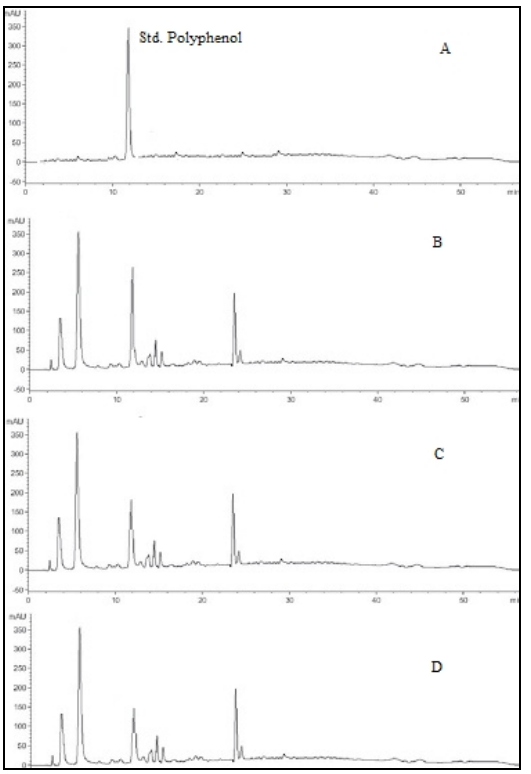

Figure 3: HPLC profile of phenolic compound: (A): Standards, (B): Plant sample collected in month-May, (C): Plant sample collected in month-December, (D): Plant sample collected in month-August

CONCLUSION

Seasonal variation is one of the key factors which investigated extensively by various researchers. The plant which possess antioxidant potential may be used for the treatment of various diseases such as; cancer with less side effects. The results of study suggested that production of phenolics compound increases during summer along with antioxidant potential, the fact can be established that phenolic compounds contributed significantly towards the antioxidant potential of the plant *Zanthoxylum armatum*. This result may become pathfinder for the researchers for further investigations.

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