EVALUATION OF PHYTOCHEMICAL COMPOUNDS IN STEM AND LEAF EXTRACT OF *ARTOCARPUS INTEGRIFOLIA* L. F.

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ABSTRACT: *Artocarpus integrifolia* L. F., economically an important medicinal plant because of its healing properties in treating multiple health disorders. The present study was intended to analyze anti-radical properties as well as phytochemical constituents of both stem and leaf extract of *A. integrifolia. A. integrifolia* (150 g) leaves were shade dried and extracted by double maceration technique via two polar solvent i.e. methanol and ethanol. Phytochemical screening and determination of phenolics and flavonoids was done by Folin–Ciocalteu reagent and aluminium chloride colorimetric methods. Radical scavenging activity of plant extracts were estimated by means of 2, 2-diphenyl-1-picrylhydrazyl assay. Total phenolic and flavonoid contents of both stem and leaf extract of *A. integrifolia* were assessed to determine the effect of Total phenolic content and Total flavonoid contents on the antioxidant activity of this plant. The result obtained from current study showed that both methanolic and ethanolic plant extracts showed presence of major classes of phytochemicals. Phenolic and flavonoids contents (1.39±0.00 & 0.93±0.05) were significantly higher in leaf extract as compared to stem using ethanol as a solvent. Both stem and leaf extract possessed significant scavenging potential in reference to used standard BHT and Vitamin E. Hence conclude that among the two parts tested *A. integrifolia* leaves contained maximum phytochemicals, antioxidant, phenolics and flavonoid compounds.

Keywords: *Artocarpus integrifolia*, Antioxidant activity, Phenolics, Flavonoids, Folin- Ciocalteu reagent, Aluminium chloride.

(Received 20-01-2017 Accepted 05-06-2017).

INTRODUCTION

Medicinal plants are rich source of secondary metabolites which are responsible for their defensive action against multiple disorders (Karadeniz *et al*., 2015; Kamiloglu *et al*., 2014; and Aiyegoro and Okoh, 2010). Phytochemicals rich Medicinal plants play an important role in treating multiple health disorders (Skrovankova *et al*., 2012).

Artocarpus integrifolia belongs to the family Moraceae and is considered as ethnomedicinally important plant because it has both antibacterial, antifungal, hyperglycemic, anti-oxidant and immunomodulatory properties (Gupta and Chaphalkar, 2015).

Present study was conducted to estimate qualitative and quantitative detection of phyto-compounds, *in vitro* antioxidant potential in both stem and leaf extract of *A. integrifolia*.

MATERIALS AND METHODS:

Aerial plant parts i.e. stem and leaves of *Artocarpus integrifolia* were collected from Jinnah Garden, Lahore in the months of Feb -May (2016) authenticated and deposited in the Prem Madan Herbarium of Lahore College for Women University, Lahore with the following Accession no: LCWU-15-114.

Preparation of Plant Extract: *A. integrifolia* leaves and stem were shade dried and macerated with two polar solvents i.e. methanol and ethanol for 3-4 days and filtered through a filtered paper (125mm) and dried by rotary evaporator at a temperature of 45°C (Malik *et al*., 2012).

Phytochemical constituents: Different tests were conducted to identify the presence and absence of diverse phytochemicals such as terpenoids (Indumathi *et al*., 2014), alkaloids (Joshi *et al*., 2013; and Abdullahi *et al*., 2013), tannins (Kannan *et al*., 2015), phenolics (Tiwari *et al*., 2011) and flavonoids (Umesh *et al*., 2010).

Terpenoids: Presence or absence of terpenoids in extracts was detected by Salkowski test. Took Quantify amount of tested material along with chloroform plus concentrated H_2SO_4, reddish brown coloration was formed at the junction which indicated the presence of terpenoids (Indumathi *et al*., 2014).

Alkaloids: Different reagents were used for the detection

of Alkaloids. In case of Mayers reagent creamish precipitate was formed, Dragondroffs reagent gave orange precipitate while brown precipitate appearance was reflected with Wagners reagent (Abdullahi *et al*., 2013; and Joshi *et al*., 2013).

Tannins: In the $FeCl_3$ test transformation of blue or greenish-black color to olive green color on progressive addition of $FeCl_3$ which represented the presence of tannins in the compound under observation. Blue, green or even red color was the indication of phenolics (Kannan *et al*., 2015).

Flavonoids: Alkaline Reagent test was used for the detection of flavonoids. Addition of 10% NaOH solution, 1% KOH, aluminum chloride to the tested compound resulted in the formation of yellow color indicating the presence of flavonoids (Umesh *et al*., 2010).

Phenolics: 2 mL of fresh leaf extract when mixed with 5% $FeCl_3$ gave blue coloration which was the clear indication of the presence of phenolics (Tiwari *et al*., 2011).

Antioxidant activity: Antioxidant assay was performed on methanol fractions of selected plant materials at four different concentrations i.e. 0.125, 0.25, 0.5 and 1mg/mL. All the fractions were incubated for half hour at 37 ^{o}C and then absorbance was measured at 517nm using blank. The color of all the tested fractions changed from deep-violet to light yellow which indicated the presence of antioxidants in all the tested fractions (Erasto *et al*., 2004).

Butylated Hydroxy Toluene and Vitamin E were used as positive control and the values were compared with the decrease in absorbance in the tested fractions. Mean and standard deviation was calculated for each fraction using SPSS software. The half maximal inhibitory concentration (IC50) was determined for each fraction using Graphpad Prism software 5.04

Total Phenolic Content: Total phenolic contents (TPC) in the methanolic and ethanolic stem and leaf extracts of *A. integrifolia* were determined by Folin Ciocalteu reagent assay using Gallic acid as a standard (Cliffe *et al*., 1994; Chlopicka *et al*., 2012).

Total Flavonoid Content: Aluminum chloride method was used for the detection of total flavonoid content using quercetin as a standard. After incubation at room temperature O.D values of samples were measured at 510 nm and expressed as mg quercetin equivalents (QE)/g fresh weight (Dewanto *et al*., 2002; Stankovic, 2011).

Statistical Analysis: Statistical analysis was done in order to study the significance of the experiment. All experiments were carried in triplicate, mean and standard deviation was calculated with SPSS software while IC50 value was calculated by Graphpad prism software 5.04. All the values attained as $p<0.05$ were deliberated as statistically noteworthy by SPSS software (Levesque, 2007).

RESULTS AND DISCUSSION

Phytochemical Screening: Different chemical test were conducted to analyze the presence or absence of diverse phyto-compounds in both stem and leaf extracts of *A. integrifolia* via two polar solvents (ethanol and methanol). Results obtained from Qualitative Analysis of Phytochemicals showed the presence of terpenoids, alkaloids, flavonoids, phenolics and tannins in the stem and leaf extract of *A. integrifolia*.

The quantitative analysis for the detection of antioxidants was done by 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity. The results obtained from the current study showed that leaf extract of *A. integrifolia* had maximum percentage of scavenging (89±0.68) at the concentration of 1mg/mL followed by (82.33±0.65) at a concentration of 0.5 mg/mL, while (78.89±0.89) at 0.25mg/mL concentration, however minimum inhibition was recorded at 0.125mg/mL (70.44±0.41) as compared to the stem extract (Fig-I). The results clearly indicated that there was a significant decline in percent radical scavenging activity with progressive decrease in the concentration which showed dose dependent inhibition.

The current results were compared with study conducted by (Sannigrahi *et al*., 2010; Sultana *et al*., 2007; and Dinis *et al*., 1994) who studied the radical scavenging potential of *P. acerifolium* leaves and observed high contents of phenolic compounds which was responsible for the scavenging potential of extracts.

DPPH scavenging activity was significantly altered by the presence of different secondary metabolites such as tannins, terpenoids, alkaloids flavonoids/anthocyanins and vitamins reported by (Chen *et al*., 2013).

The IC50 was also calculated for both stem and leaf extracts of *A. integrifolia* L. by Graphpad prism software 5.04. Stem extract showed 0.91±0.00 while leaf extract showed IC50 value of 0.03±0.00.

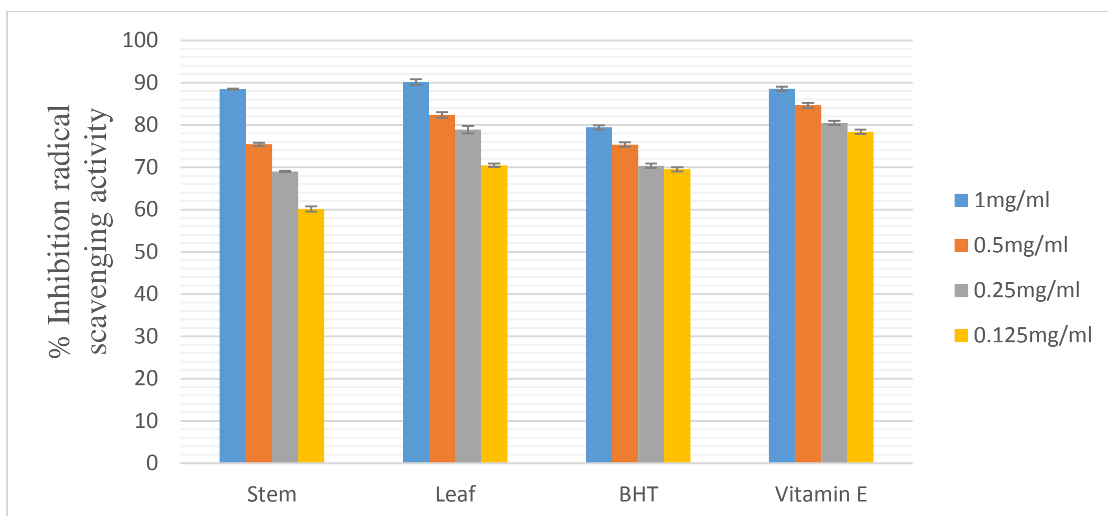

Fig-I Anti-radical scavenging activity of leaf and stem extract of *Artocarpus integrifolia* L. F.

Total Phenolic Content: The amount of total phenolics in ethanolic leaf extract was 1.06±0.05 as compared to the methanolic leaf extract i.e. 0.87±0.05. Total phenolic contents were significantly higher in the methanolic and ethanolic leaf extract in comparison to the stem extract using Gallic acid as standard. The results correlate to the findings of Amoo *et al*., (2012) that the phenolic and flavonoid contents vary significantly from tissue to tissue and higher amount of TPC and TFC was reported in the leaves than that in the tubers and stems.

Fig-II (a) illustrates the amount of phenolic content present in stem and leaf extracts of *Artocarpus integrifolia*. Fig-II (b) clearly indicates the Caliberation curve for Gallic acid. Maximum phenolic and flavonoids contents were observed in the ethanol solvent. The reason behind this was the polarity of the ethanol which resulted in the better extraction of phenolics and flavonoids from different plant parts i.e leaves, stem and tubers etc reported by (Vamanu *et al*., 2011). Maximum amount of Phenolic compounds was detected in leaves (Oliveira *et al*., 2009). Variations in the distribution of phenolic and flavonoids contents were observed at both intercellular and intracellular level. The findings correlate with the work of different researchers (Akond *et al*., 2010; Carlsen *et al*., 2010; Sannigrahi *et al*., 2010; Mullen *et al*., 2007; and Randhir *et al*., 2000).

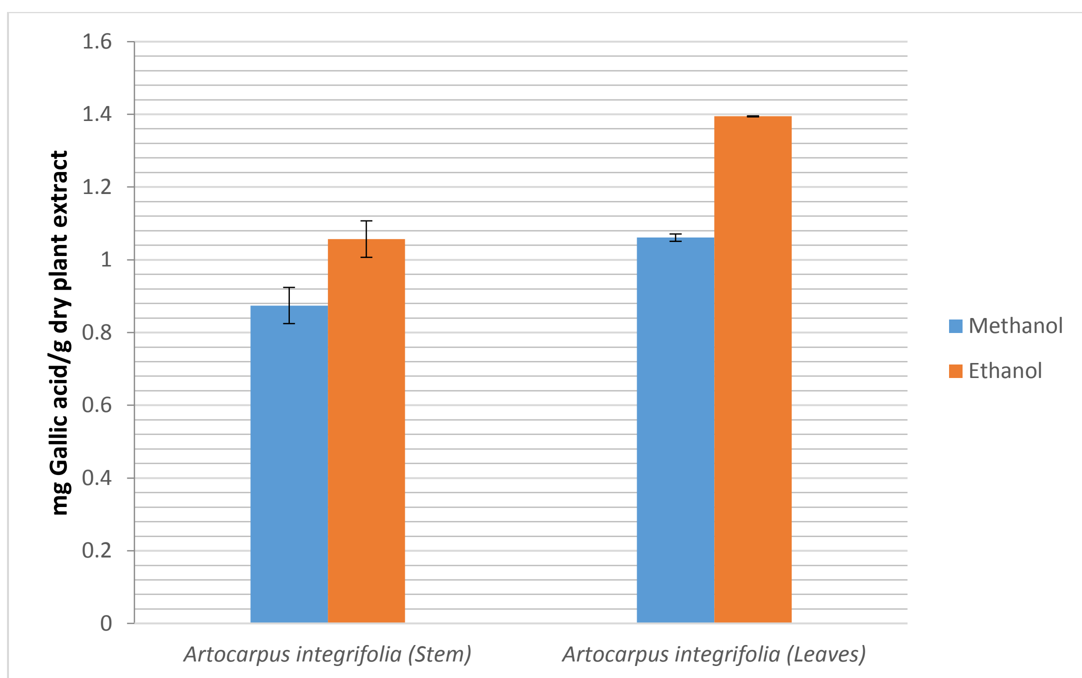

Fig-II (a): Total phenolic content (mg Gallic acid/g dry plant extract)

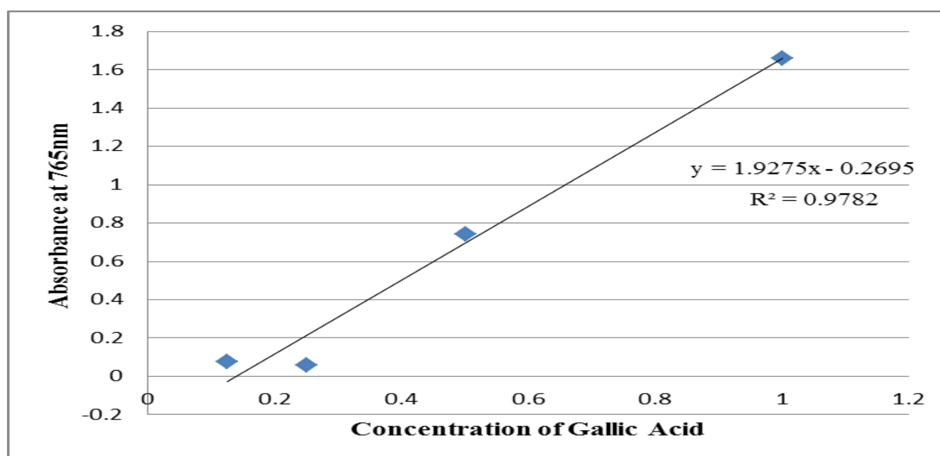

Fig-II (b): Caliberation curve for Gallic Acid

Total flavonoid Contents: Total flavonoids obtained from ethanolic leaf extracts were higher (0.93± 0.05 mg QE/ g of dry extract) as compared to the ethanolic stem extract (0.66±0.07). Fig-III (a) which clearly indicated the amount of flavonoids content present in stem and leaf extract of ethnomedicinally important plants i.e. *Artocarpus integrifolia* L. Fig-III (b) illustrate the Caliberation curve for Quercetin

The findings showed a positive correlation among TPC, TFC and antioxidant activity which were in agreement with the findings of Ghasemzadeh *et al*. (2012). Similarly a strong correlation existed between the phenolic contents and the antioxidant capacity as reported by (Çalişkan and Polat, 2011; Oliveira *et al*., 2009; Veberic *et al*., 2008; and Konyalıoğlu *et al*., 2005).

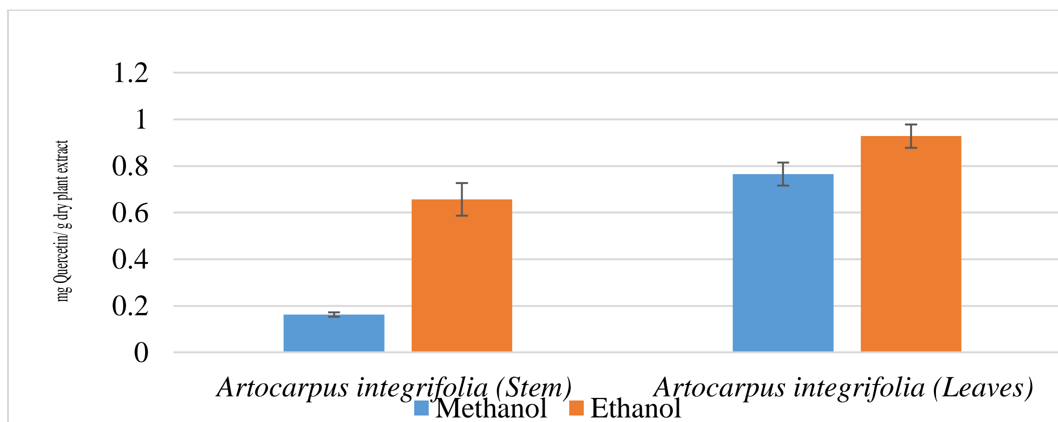

Fig-III (a) TFC (mg Quercetin/ g dry plant extract)

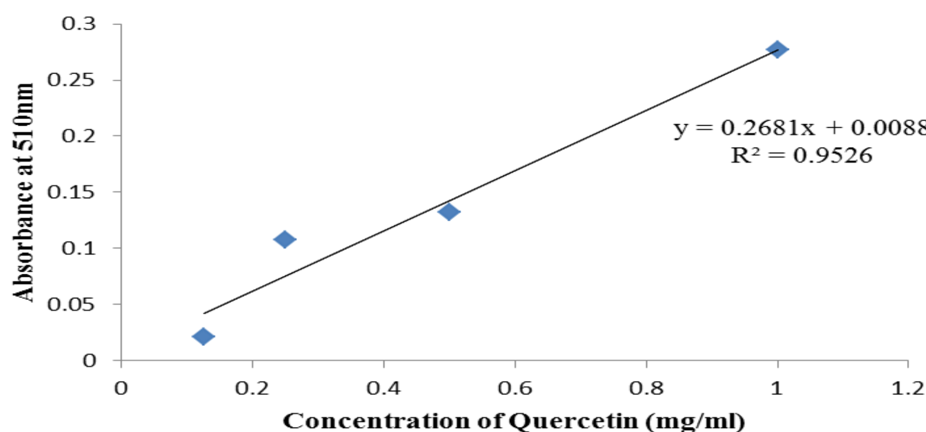

Fig-III (b) Caliberation Curve of Quercetin (mg/mL)

Conclusion: Hence, conclude that *A. integrifolia* possessed significant antioxidant potential because of the presence of flavonoids and phenolics content and maximum anti-radical activity was detected from ethanolic leaf extract as compared to the stem extract of *A. integrifolia*. Hence this plant can be used as a source of natural antioxidant in comparison to the artificial/manmade standards.

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