

COMPARATIVE PROFILING OF PHYTOCHEMICAL CONTENTS AND RADICAL SCAVENGING POTENTIAL IN SOME SPECIES OF FAMILY MORACEAE

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ABSTRACT: The current study was carried out for comparative estimation of phytochemicals, anti-radical properties and half maximal inhibitory concentration (IC₅₀) of seven species of family Moraceae using 2, 2-Diphenyl-1-Picrylhydrazyl assay. Methanolic (70%) extraction of crude leaves extracts was done via Double Maceration technique. Quantitative assay for determining the radical scavenging activity of DPPH as well as IC₅₀ value of seven species of family Moraceae was observed. Results showed the presence of all major classes of phyto-compounds in seven species of family Moraceae, maximum percent inhibition radical scavenging activity was observed by *Ficus infectoria* (96±0.21) while minimum in *Ficus macrophylla* (scavenging >79.08%). Half maximal inhibitory concentration (IC₅₀) of all the seven species of family Moraceae was done by Graph pad prism 5.04 software. Concentration dependent percent inhibition was observed in all the species of Family Moraceae. However all the selected species had significant antioxidant potential which indicated the pharmaceutical uses of all the species under study on commercial scale in near future.

Keywords: DPPH, Radical scavenging activity, Maximal inhibitory concentration and Moraceae.

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INTRODUCTION

Medicinal plants play an important role in the prevention and cure of diseases in comparison to conventional treatments that are injurious to health (Bachrach, 2012). Plants are enriched source of secondary metabolites having biological and pharmacological activities (Atanasov *et al.*, 2015).

Natural Antioxidants possess significant potential in the wellbeing of human beings by strengthening defensive mechanism in the prevention of prolonged syndromes like cancer and cardiac diseases (Pisoschi and Pop, 2015). Antioxidant compounds include flavonoids, phenolic acids, carotenoids, and tocopherols that can inhibit Fe³⁺/AA induced oxidation, scavenge free radicals, and act as reductants (Ozsoy *et al.*, 2009).

Moraceae family consists of 37 genera and 1,050 species including ethnomedicinally important species like *Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg., *Broussonetia papyrifera* Vent. and *Ficus* L. All the species are dispersed all over humid and mild regions worldwide; however diversity is centered in the tropics. *Ficus lyrata* has the potential to treat Gastrointestinal problems, anthelmintic, diabetes, antitumor activity, asthma, cough, sexual disorders, diarrhea, ear-ache and toothache, migraine, eye troubles, scabies, gonorrhea, bleeding, paralysis, bone fracture, antiseptic and astringent (Nisar *et al.*, 2014). *Artocarpus lakoocha* has pharmacological activities including anti-inflammatory,

antiviral, anticancer and anti-HIV (Hossain *et al.*, 2016). *Ficus retusa* leaves and bark are used for rheumatism, abdominal colic, flatulence, liver diseases and rootlets for toothaches (Ravichandra *et al.*, 2011; and Sirisha *et al.*, 2010). *Ficus elastica* is an important reagent in the cure of dermatological issues and possess both antibacterial and antifungal activities (Ogunwande *et al.*, 2011). *F. macrophylla* leaves possess medicinal potential for the therapy of bacterial infections induced by *S. aureus* (Tkachenko *et al.*, 2016). *Ficus virens* bark methanolic (FVBM) extract contained large amount of antioxidant with significant hypolipidemic property (Iqbal *et al.*, 2014b; and Iqbal *et al.*, 2015). *Ficus infectoria* is used in many parts of the world for the treatment of a number of diseases, e.g. as an antibacterial, antifungal and hyperglycaemic (Chandira *et al.*, 2010).

DPPH is the easy and rapid approach for assessing the free radical scavenging activity of various products at a time by Colorimetric detection method. Chemistry behind the process is the reduction of stable free radical DPPH. Discolorization is reflected which is a clear indication of the fact that the compound possessed reducing ability hence an effective way for analyzing the antioxidant potential of different novel compounds by spectrophotometry (Chen *et al.*, 2013).

The current study was carried out to analyze the qualitative estimation of various phyto-compounds as well as antioxidant potential of all the seven species of Family Moraceae, as assessed using DPPH assay.

MATERIALS AND METHODS

Fresh parts Leaves /Fruit of seven species of Family Moraceae 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 (*Artocarpus lakoocha* Wall. ex Roxb. Voucher No: LCWU-15-104, *Ficus lyrata* Warb. Voucher No: LCWU-15-117, *Ficus elastica* Roxb. ex Hornem Voucher No: LCWU-15-119, *Ficus macrophylla* Desf. ex Pers. Voucher No: LCWU-15-105, *Ficus virens* Aiton. Voucher No: LCWU-15-113, *Ficus retusa* L. Voucher No: LCWU-15-126, *Ficus infectoria* Auct. Voucher No: LCWU-15-103).

Preparation of Plant Extracts: Plant material washed with sterilized water, dried and milled into fine powder. Extracts were obtained using methanol by double maceration with continuous stirring for a week. The resulting crude extracts were filtered through a Whatman filter paper. This process repeated twice or thrice followed by dryness in rotary evaporator at 40°C (Malik *et al.*, 2012).

Phytochemical Screening: Phytochemical screening was performed for terpenoids, alkaloids, tannins, flavonoids and phenolic acids.

Terpenoids: Salkowski test was used for determining the presence and absence of terpenoids in extracts. Took Quantify amount of tested material along with chloroform plus concentrated H₂SO₄ reddish brown coloration was formed at the junction indicating the presence of terpenoids (Indumathi *et al.*, 2014).

Alkaloids: The presence, absence of alkaloids was estimated with the help of Mayers reagent, Dragondroffs reagent and Wagners reagent. In case of Mayers reagent creamish precipitate was formed, Dragondroffs reagent gave orange precipitate while brown precipitate appearance was reflected with Wagners reagent (Joshi *et al.*, 2013 ; and Abdullahi *et al.*, 2013).

Tannins: In the FeCl₃ test transformation of blue or greenish-black color to olive green color on progressive addition of FeCl₃ representing the existence of tannins in the compound under observation. On the basis of phenol present variations in the color from blue, green or even red was demonstrated (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 was taken along with 5% FeCl₃ as a result deep blue coloration was observed which was the clear indication of phenolics

(Tiwari *et al.*, 2011).

DPPH free radical scavenging activity: Antioxidant assay was performed on methanol fractions of selected plant material at four different concentrations (0.125, 0.25, 0.5 and 1mg/mL). All the fractions showed significant antioxidant activity at all the concentrations. Plant extracts (50µL) was added in methanol solution of DPPH (5ml of 0.05mM) at four different concentrations i.e. 0.125, 0.25, 0.5 and 1mg/mL. The color of all the tested fractions changed from deep-violet to light yellow that indicated the presence of antioxidants in all the tested fractions.

BHT and Vitamin E were used as positive control and values were compared with the decrease in absorbance in the tested fractions. Half maximal inhibitory concentration means 50% inhibition was determined for each fraction (Erasto *et al.*, 2004).

Statistical analysis: Statistical analysis to calculate the significance of the experiment using SPSS software while IC50 value was calculated by Graphpad prism software 5.04 (Chen *et al.*, 2013).

RESULTS AND DISCUSSION

The current experiment was conducted to determine the phyto-compounds, antioxidant potential as well as the half maximal inhibitory concentration of seven species of Family Moraceae. Qualitative estimation of major classes of Phytochemical constituents in all seven species of Family Moraceae revealed the presence of terpenoids, phenolics, flavonoids and tannins however absence of alkaloids was reflected in three species under observation including *Artocarpus lakoocha*, *Ficus lyrata* and *Ficus virens* (Table 1). The existence of phyto-compounds indicated the antioxidant and anti-inflammatory activities of all these species hence could be used as antimicrobial. This also correlated to the work of different researchers reporting that phyto-compounds isolated from plant origin have antiradical, anti- bacterial, sarcoma and anti-fungal activities (Hossain and Nagooru, 2011; and Suresh and Nagarajan, 2009).

Free radicals lead to Oxidative stress causing drastic disorders like brain related syndromes, sarcoma, AIDS and so on. Antioxidants play a defensive mechanism in the prevention of multiple diseases because of the ability to scavenge free radicals. One of the easy approach for determining the radical scavenging potential of plant extract is DPPH assay. DPPH assay is a unique, active, modest and reproducible *in vitro* method for assessing vital activity of one or more than one compound as well as plant extracts (Bhat *et al.*, 2016).

Figure I show the concentration dependent response curve of DPPH scavenging activities of the crude methanolic extract in all the seven species of Family Moraceae. The antioxidant activity of all seven

species of Family Moraceae was compared with Vitamin E and BHT as standard references. Free radicals were involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants due to scavenging activity are useful for the management of diseases. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts (Bhat *et al.*, 2016). Figure II shows the amount of each extract required for 50% inhibition of DPPH activity (IC50). The free radical scavenging action of methanol extracts of plant are in the order as *Ficus infectoria* > *Ficus elastica* > *Ficus lyrata* > *Artocarpus lakoocha* > *Ficus virens* > *Ficus retusa* > *Ficus macrophylla* (Fig I).

However, IC50 value of seven species of family Moraceae was calculated using Graph Pad prism software 5.04. *Artocarpus lakoocha* showed highest IC50 value of 0.52 ± 0.01 while least value was reflected by *Ficus virens* 0.09 ± 0.00 . Following results were illustrated in the Fig II.

Many medicinal plants with antioxidant properties possess therapeutic potential in curing different diseases. So all the species of family Moraceae studied so far have the potential to attenuate oxidative stress due to anti-radical properties and hence helped in the prevention of multiple disorders i.e. neurological disorders, Cancer and so on (Ozkan *et al.*, 2016).

Table 1: Phytochemical screening of seven species of Family Moraceae.

Plant species	Results				Tannins
	Terpenoids	Alkaloids	Flavonoids	Phenolics	
<i>Artocarpus lakoocha</i>	++	--	++	++	++
<i>Ficus lyrata</i>	++	--	++	++	++
<i>Ficus elastica</i>	++	++	++	++	++
<i>Ficus macrophylla</i>	++	++	++	++	++
<i>Ficus virens</i>	++	--	++	++	++
<i>Ficus retusa</i>	++	++	++	++	++
<i>Ficus infectoria</i>	++	++	++	++	++

+ Presence, ++ Maximum Presence, - Absence

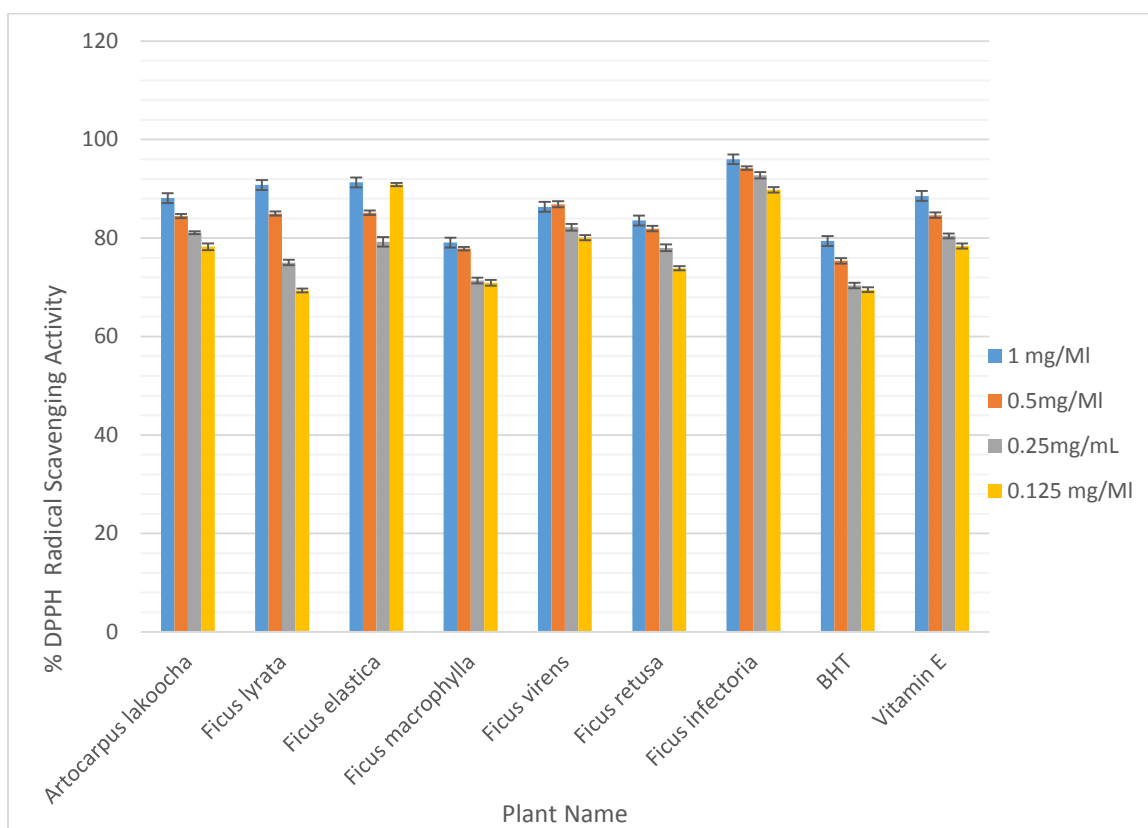


Fig 1: Anti-radical scavenging activity of 7 medicinally important species of family Moraceae in comparison of known standards

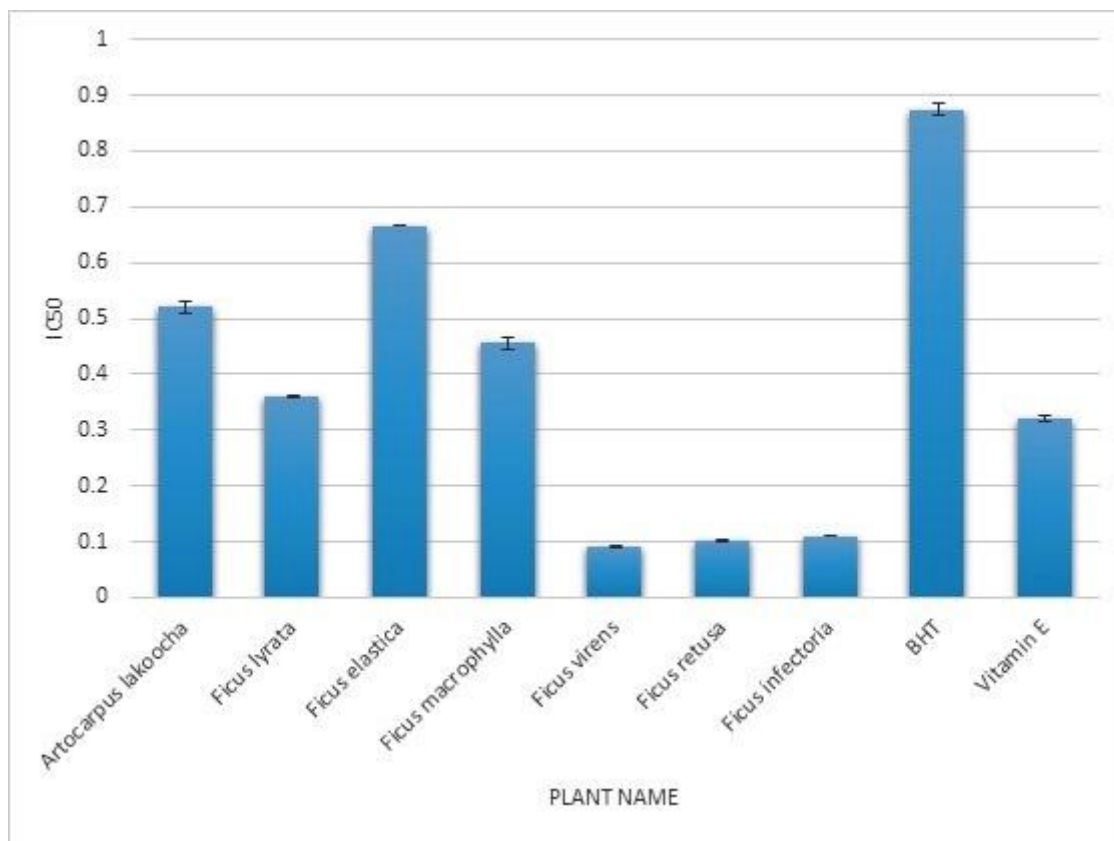


Fig 2: Half maximal inhibitory concentration (IC50) in seven species of Family Moraceae using Graphpad prism software 5.04.

Conclusion: Among the seven species used *Ficus infectoria* had significant antioxidant activity as compared to the used standards i.e BHT and Vitamin E for DPPH radical scavenging activity and might be used as a substitute to the man-made antioxidants.

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