

## INVESTIGATION OF *IN-VITRO* ANTIOXIDANT POTENTIAL OF ETHNOBOTANICALLY IMPORTANT TREE, *PTEROSPERMUM ACERIFOLIUM* L.

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### Abstract

The present study was based on the investigation of an ethnopharmacological activity, i.e. antioxidant potential of a traditionally used important tree, *Pterospermum acerifolium* Linn. The crude extracts of fruit, leaf, stem and bark were extracted in polar & non polar solvents, viz: petroleum ether, chloroform, methanol and water by maceration.

Antioxidant profile of *P.acerifolium* Linn. was evaluated by three methods: scavenging of DPPH, total antioxidant assay along with the determination of total phenolic contents. Results revealed that *P.acerifolium* Linn., had significant antioxidant DPPH scavenging potential (scavenging >80%) and total antioxidant contents >0.562, especially its fruit, stem and bark showed very good antioxidant potential. Moreover, total phenolic contents were also higher in fruit, stem and bark extracts, i.e., 0.149, 0.124 and 0.137 mg of Gallic acid equivalents (GAE) respectively. The antioxidant results were quite reasonable as compared with the standard antioxidants ( $\alpha$ -tocopherol, vitamin E and BHT).

### Introduction

*P. acerifolium* Linn., has a wide application in traditional system of Indian medicine for example, in ayurvedic anticancer treatment flowers are mixed with sugars and applied on skin (Balachandran, 2005). Flowers and bark, charred and mixed with kamala, applied for the treatment of smallpox. Flowers made into paste with rice water is used as application for hemicranias (Caius, 1990). Flowers are considered to be laxative, anthelmintic, anti-inflammatory, and controls many blood disorders, ulcers and leprosy due to antioxidant contents (Sannigrahi *et al.*, 2010). Previously, the pharmacognostic properties of flower have been studied to justify its use in folkloric medicines (Mehrotra & Shone, 2008).

Plants have been used by rural and tribal people to cure diseases, new resources for pharmacology, herbal drugs, food, and other aspects of life (Jain & Mudgal, 1999). Today, almost 80% of the population of the world countries including Pakistan still relies on the indigenous system of herbal medicine, known as ethnopharmacology (Anon., 1996).

Synthetic and natural antioxidants have been used to prevent food and living systems from peroxidative damage. However, in the recent years more research has been done to find out natural antioxidants to control disorders such as cancer and cardiovascular diseases from plants because they are not having disadvantages like BHT and other synthetic antioxidants, hence replacing them very fast (Valentao *et al.*, 2002; Sagi & Fluhr, 2006; Shcolnick & Keren, 2006).

The aim of the present study was to investigate the antioxidant activity of various extracts of fruit, leaves, stem and bark of *Pterospermum acerifolium* by using three antioxidant assays.

### Material and Methods

*Pterospermum acerifolium* Linn., (Sterculiaceae) is commonly known as Kanack Champa and Moo Chkund. It is a large tree, up to 30m tall, with white gray bark and rusty pubescent young parts. Leaves are oblong, broad and wavy margined. Flowers are mostly solitary.

Flowering period lasts from March to May (Abedin & Ghafoor, 1976).

Aerial parts (fruits, leaves, stem and bark) of *Pterospermum acerifolium* were collected from GCU Botanic Garden and Jinnah Garden, Lahore in the months of March-May (2010). Voucher specimens (GC.Bot.Herb.955) were deposited in Dr. Sultan Ahmad Herbarium, GC University Lahore, after authenticating by Dr. Zaheer-ud-din Khan, Professor of Botany, GC University, Lahore.

**Plant material and extracts:** The fruit, branches, leaves and bark were dried at room temperature, i.e., 21°C, and were crushed to coarse powder. About 150g of dried powder was extracted successively by maceration for 3-4 days each petroleum ether (40-60C), chloroform, methanol and distilled water (Inmaculada *et al.*, 2005).

**DPPH free radical scavenging activity:** Radical scavenging activity of plant extracts (0.5mg/ml) was established by measuring the decrease in absorbance of 2, 2-diphenyl- 1-picrylhydrazyl (DPPH) radical according to Erasto *et al.*, (2004). Equal volume (2.0 ml) of methanolic extracts (0.5 mg/ml) and reference solution [0.5mg/ml, BHT and Vitamin E] were mixed in test tubes and incubated at 30°C for 15 min in dark. Control solution containing equal volume of DPPH and methanol was used as blank ( $A_{\text{blank}}$ ). Absorbance was measured at 517 nm to avoid the inaccurate data (excess values) caused from the absorbing materials without antioxidant activity, which can exist in extracts. Actual absorbance ( $A_{\text{sample}}$ ) originated from the inhibition of DPPH ( $A_{\text{sample}}$ ) was evaluated by subtracting the absorbance of methanolic extracts (or reference compounds), from absorbance of corresponding DPPH extracts (or reference compound) at 517 nm. Decrease in absorbance indicated the antioxidant activity. Radical scavenging activity was expressed as percentage inhibition of DPPH and estimated by the following formula:

$$\% \text{ Inhibition of DPPH} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$

**Total antioxidant content (TAOC):** The total antioxidant capacity of all the extracts was assayed according to the method of Prieto *et al.*, (1999). Stock solutions of BHT and  $\alpha$ -tocopherol were prepared in methanol and kept at 20°C. 0.1 ml of sample solution (0.5mg/ml) in methanol was combined in test tube with 1.9 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 1.9 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples.  $\alpha$ -tocopherol and BHT (Butylated hydroxytoluene) were used as standards for comparison.

**Total phenolic contents:** Total phenolics of different parts of plants were determined by the method of Makkar *et al.*, (2006). Approximately 0.1 ml of the sample (0.5 mg/ml) was combined with 2.8 ml of 10% Na<sub>2</sub>CO<sub>3</sub> and 0.1 ml of 2.0 N Folin-Ciocalteu reagent in test tubes. After 40 min absorbance at 725 nm was measured by UV-visible spectrophotometer. Total phenols were determined as milligrams of gallic acid equivalents (GAE) per gram of extract by computing with standard gallic acid graph constructed for different concentrations of gallic acid. All samples were analyzed in triplicates.

## Results and discussions

% DPPH free radical scavenging activity of various extracts of fruit, leaves, stem and bark of *P.acerifolium*, was measured (Fig. 1a-d). The results provided a direct comparison of the antioxidant activities with standard antioxidants, i.e. BHT (77.3%) & Vitamin E (88.1%). The petroleum ether extract of bark demonstrated the highest % scavenging (88.54%) followed by methanolic extract of leaf (80.25%) and petroleum ether extract of leaf (73.09%), while remaining extracts were almost active and showed a broad range of (30-70%) scavenging of DPPH radical (Fig. 1 a-d). This work agrees with the findings of Dinis *et al.*, (1994); Sultana *et al.*, (2007) and Sannigrahi *et al.*, (2010) who determined antioxidant potentials of leaves of *P. acerifolium* for reducing oxidative stress. Antioxidant property of the extracts was due to the high contents of phenolic compounds (Fig. 3a-e) that react differently with DPPH or may be because of their synergistic effect. The presence of a diverse array of secondary metabolites such as tannins, terpenoids, alkaloids, vitamins (Head, 1998), flavonoids/anthocyanins (Pourcel *et al.*, 2007) may also affect DPPH scavenging activity due to difference in terms of structure and biological properties. Furthermore, flavan-3-4-diol (fustucacidin) is usually the major component identified in plant barks and it expected donation of hydrogen from it to quench DPPH radicals. However, the underlying mechanism may not involve hydroxyl radical scavenging property.

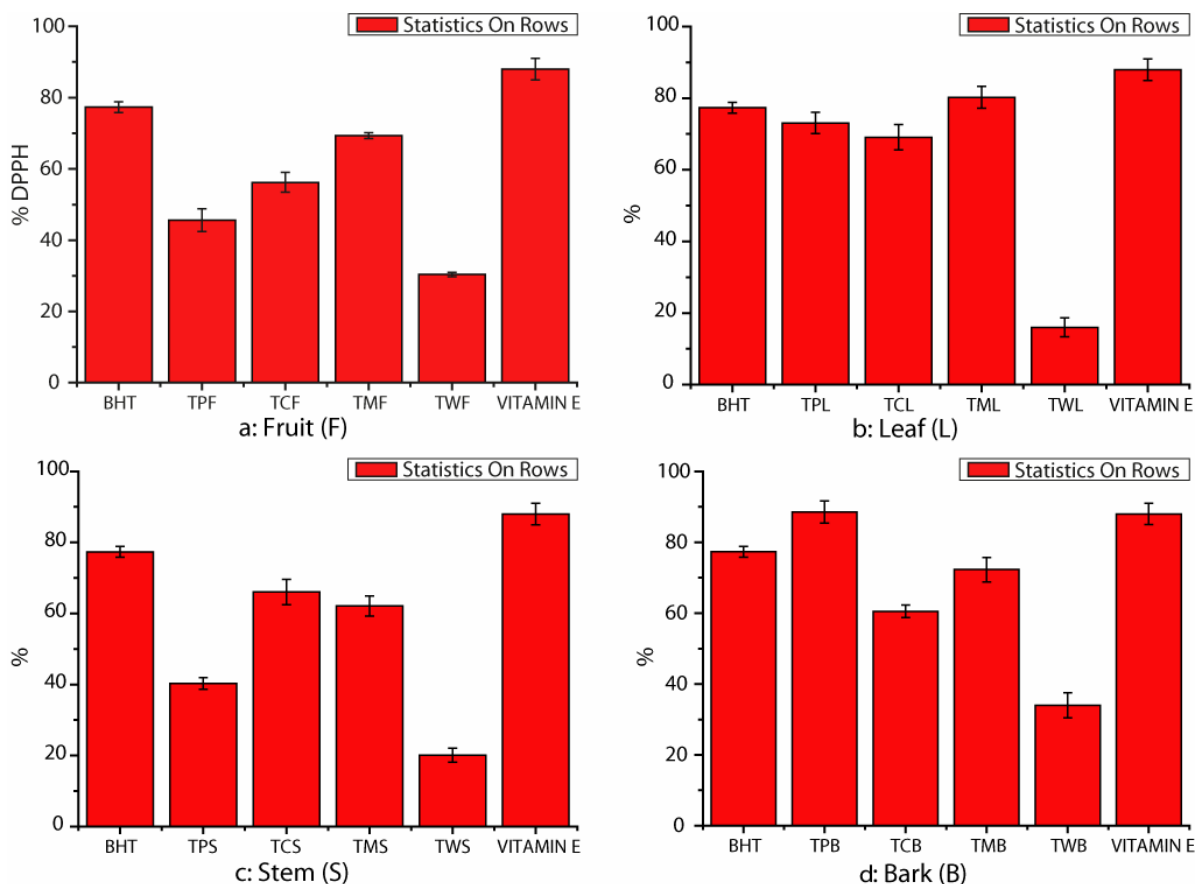


Fig. 1. a-d: DPPH Scavenging free radical activity of different parts of *P.acerifolium*, TP (*P.acerifolium* petroleum ether), TC (*P.acerifolium* chloroform), TM (*P.acerifolium* methanol), TW (*P.acerifolium* distilled water).

TAOC assay determines the reducing ability of the antioxidants through the generation of green phosphate/Mo(V) complex formed by the reduction of Mo(VI) to Mo(V). Petroleum ether extract of fruit exhibited the highest absorbance; 0.56 at a concentration of 0.5 mg/mL comparable with the standard BHT (absorbance = 0.47 at 0.5 mg/mL) &  $\alpha$ -tocopherol (absorbance = 0.51 at 0.5 mg/mL). The total antioxidant activity was measured and compared with that of BHT and  $\alpha$ -tocopherol. The high absorbance values indicated that all the extracts, possessed significant antioxidant activity (Fig. 2a-d). However, petroleum ether extract of fruit showed maximum total antioxidant potential (Fig. 2a) indicating that maximum amount of antioxidants are present in fruit of *P. acerifolium*, but total antioxidant assay does not identify single antioxidant compounds. It

was also noticed that the total antioxidant activity of some other parts of *P. acerifolium* was comparatively higher than those of the standard antioxidants, like BHT and  $\alpha$ -tocopherol. This indicated the ethnomedicinal importance of *P. acerifolium* and proved it a good antioxidant agent. These findings can be supported by Sultana *et al.*, (2007); Carlsen *et al.*, (2010) and Akond *et al.*, (2010). High value of antioxidants was due to the presence of reactive oxygen species, which may be produced by metabolic reactions, such as photosynthesis, photorespiration and respiration. However, the presence of predominant conjugated antioxidants, hydroxycinnamic acids, alkaloids, flavonoids and anthocyanins may affect total antioxidant values (Carlsen *et al.*, 2010 and Akond *et al.*, 2010).

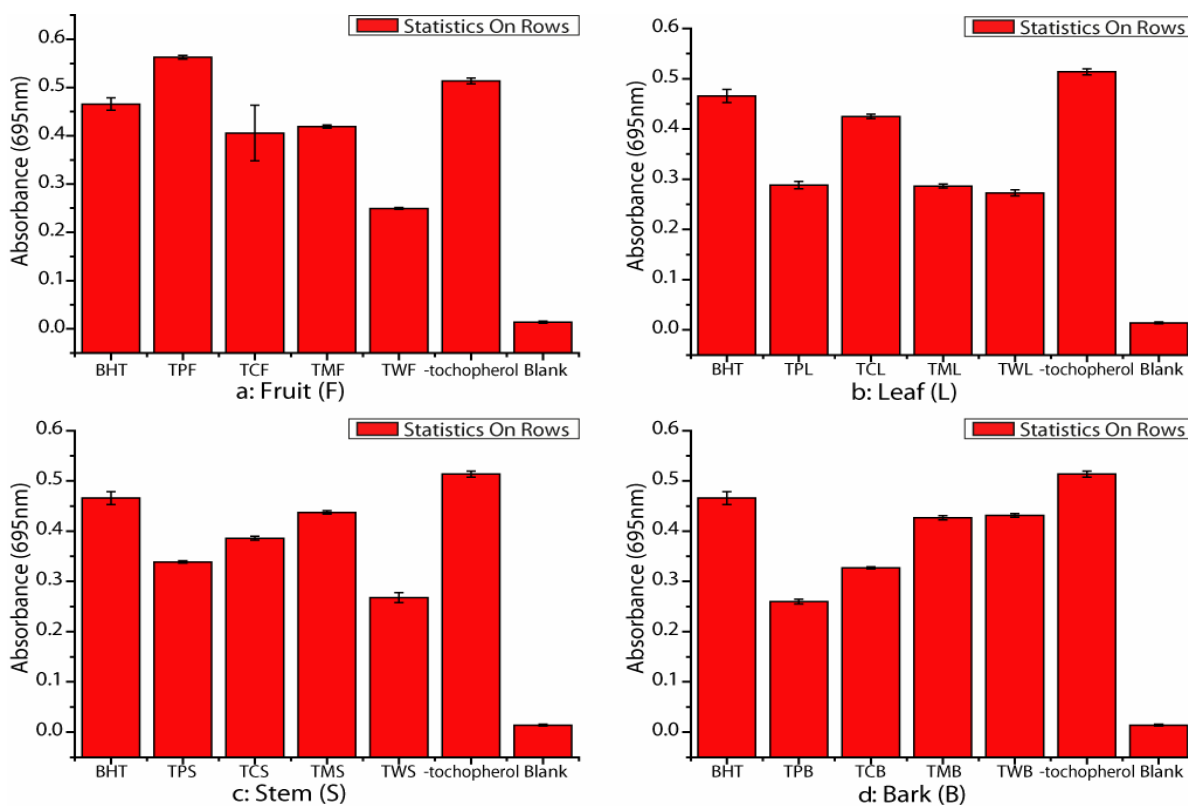


Fig. 2. a-d: Total Antioxidant Assay of different parts of *P. acerifolium*, TP (*P. acerifolium* petroleum ether), TC (*P. acerifolium* chloroform), TM (*P. acerifolium* methanol), TW (*P. acerifolium* distilled water).

Total phenolic contents of all extracts of various parts of *P. acerifolium* were determined as milligrams of gallic acid equivalents mg GAE/ 0.5mg of extract (Fig. 3 a-d), by computing with standard calibration curve constructed for different concentrations of gallic acid (Fig. 3 e). The result revealed that the amount of total phenolics ranged from 0.09 to 0.149 (mg GAE/ 0.5mg of extract) for various parts of *P. acerifolium*. The antioxidant activity of plant origin components can mainly be ascribed to the presence of phenolic compounds (Heim *et al.*, 2002). The results in the present study also showed that phenolic contents were not evenly distributed in plant parts hence their amount varied in fruit, leaves, stem and bark. Among all parts, Chloroform extract of *P. acerifolium*

stem (Fig. 3c) revealed the highest phenolic contents, i.e., 0.149 (mg GAE/ 0.5mg of extract). This may be because of the polarity of the chloroform showed the best effectiveness in extracting phenolic compounds from the plant parts (Tung *et al.*, 2007). Many studies also confirmed that composition of phenolic compounds is diversified at subcellular level and within tissues (Randhir *et al.*, 2000). These findings are in also in accordance with Carlsen *et al.*, (2010); Mullen *et al.*, (2007); Akond *et al.*, (2010) and Sannigrahi *et al.*, (2010). However, these results may also indicate the presence of polyphenolic contents, tannins, saponins and gallic acid in the various extracts of the targeted tree.

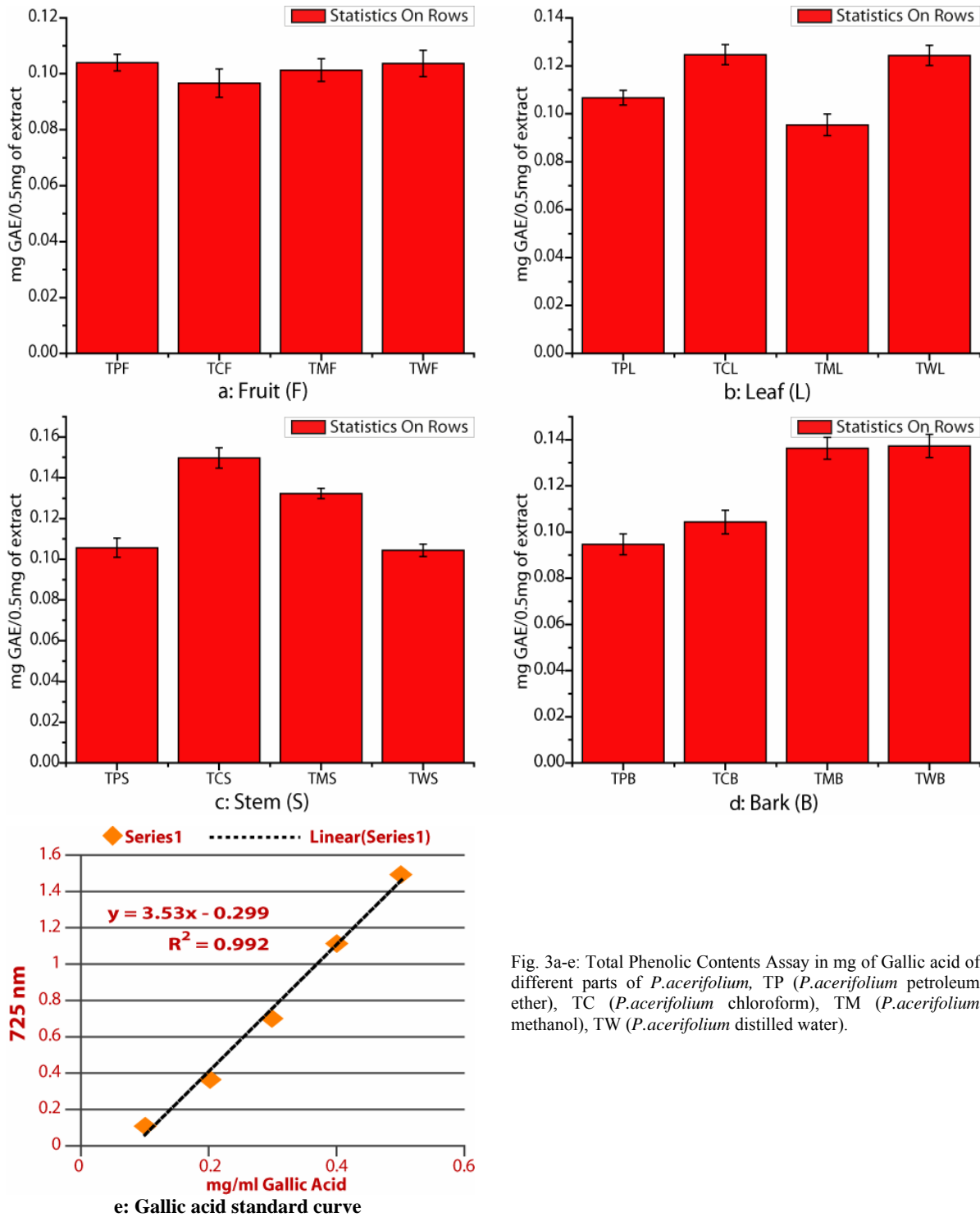


Fig. 3a-e: Total Phenolic Contents Assay in mg of Gallic acid of different parts of *P. acerifolium*, TP (*P. acerifolium* petroleum ether), TC (*P. acerifolium* chloroform), TM (*P. acerifolium* methanol), TW (*P. acerifolium* distilled water).

## Conclusion

The data presented here shows that fruit, leaves, stem and bark of *P. acerifolium* extracts, have great significant antioxidant activity almost equal to that of standards and may be used as an alternative to the synthetic antioxidants. Thus this study gives a strong impact for expanding the investigations of natural antioxidants for

use in health cares and food industry. Even though the antioxidant activity of the compounds present in different extracts was strong, the overall antioxidant effect could be higher by the combined and synergistic effects of further purified/isolated individual compounds. Therefore isolation and identification of individual active compounds, their *in vivo* antioxidant activities as well as different antioxidant mechanisms *In vitro* are still needed.

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