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Antioxidant Activity of the Extracts Derived from *Terminalia catappa*

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Abstract. The extracts derived from *Terminalia catappa* leaves and fruit following antioxidant activity directed isolation, were screened for their antioxidant activity through their ability to scavenge DPPH radicals. Only fractions which exhibited >50% DPPH scavenging effect at each step of isolation were selected for further purification and judge their ability to reduce peroxide formation (peroxide value) in heated corn oil. The results indicated that crude ethanolic extract, aqueous fraction of crude extract and its sub fractions (petroleum ether and ethylacetate) possessed prominent antioxidant activity. In addition, phytochemical analysis showed that the five fractions obtained finally contain simple phenols, anthocyanins, phenyl propanoids and flavanols.

Keywords: *Terminalia catappa*, antioxidant activity, activity directed isolation, phytochemical analysis

Introduction

The excess production of active oxygen species, such as '$OH^{\bullet}$, O_2^-, singlet oxygen and other free radicals, causes damage throughout the cell by oxidizing a variety of molecules, including unsaturated lipids. Lipids form major membrane components and their oxidation leads to significant changes in membrane properties. These changes initiate processes leading to carcinogenesis, mutagenesis, aging and arteriosclerosis (Culter, 1992; Stadtman, 1992; Pryor, 1986). Free radicals are also involved in the deterioration of food and oil (Naz *et al.,* 2005; Naz *et al.,* 2004). Cells have limited possibility in eradicating free radicals, hence it is believed that delivering endogenous antioxidants enhances its ability to protect vital biological functions (Osawa *et al.,* 1990; Kohen *et al.,* 1988; Culter, 1984); hence, there is increasing interest in the application of naturally occurring antioxidants as therapeutic agents. Fruit and vegetable antioxidants play an important role in reducing the risk of degenerative diseases, such as cardiovascular diseases, various cancers and neurological diseases (Ames *et al.,* 1993). Ascorbate is the most studied antioxidant vitamin due to its role in reducing the risk of degenerative diseases (Fraga *et al.,* 1991). However, recent studies have shown that fruit and vegetable total phenolics and anthocyanins contribute more to the antioxidant capacity than ascorbate (Connor *et al.,* 2002; Kang and Saltveil, 2002; Deighton *et al.,* 2000; Kalt *et al.,* 1999).

Terminalia catappa is very well known for its therapeutic values since long and has proved by many researchers to be useful as anti-inflammatory (Fan *et al.,* 2004; Jayasinghe *et al.,* 2000), anticancer (Kandil *et al.,* 1999), antihepatotoxic (Lin *et al.,* 2001), antigenotoxic (Chen *et al.,* 2000), anticlastogenic (Liu *et al.,* 1996) and for the treatment of skin aging, irritation, hyperpigmentation, allergy (Renimel *et al.,* 1998) and bronchial asthama in children (Prazeres, 1995). The plant also exhibits antimicrobial (Pawar and Pal, 2002), insecticidal and molluscicidal activities (Jayasinghe *et al.,* 2000).

In view of its high medicinal potential and previous findings, the study was designed to isolate various fractions from the fruit and leaves of *T. catappa* for antioxidant activity so that its further possible uses in medicine, therapy and food preservation could be determined.

Materials and Methods

Plant material. Leaves and fruits of *T. catappa* were collected in the month of February from the nursery of the University of Karachi. The sample was identified by a taxonomist of the Department of Botany, University of Karachi and a voucher specimen was

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deposited in the herbarium of the Department of Botany, University of Karachi.

Antioxidant activity directed isolation. The experiments were separately performed but similarly conducted for the fruit and leaves of *T. catappa*. Two kg fruits and leaves (each cut into small pieces) were soaked separately, in 5 L of absolute ethanol for a week. The samples were continuously stirred on a magnetic stirrer at a constant speed (1400 rpm) during the period and then filtered. The filtrates were dried on a rotary evaporator at 30 °C. The dried extract (fruit, 96.8 g and leaves, 85.4 g) was mixed and shaken thoroughly with 400 mL *n*-hexane and 400 mL distilled water in a 1/L separating funnel and then the contents were left till complete separation of the layers. Two distinct layers so formed in case of fruit sample were collected separately as F-H(*n*-hexane, upper, yellow layer) and F-W (aqueous lower, red layer) and in leaf sample as L-H and L-W. The crude ethanolic as well as fractions F-H, F-W, L-H and L-W were screened for the antioxidant activity. The fractions F-W and L-W which showed antioxidant activity were isolated further.

F-W, collected as red powdery mass (66.4 g), was mixed with 500 mL of (4:1) methanol: H_2O, homogenized and then filtered. The filtrate was evaporated to 1/10 of its original volume in a rotary evaporator at <40 °C, acidified with 2M H_2SO_4 and then extracted with 250 mL chloroform. Both the chloroform (F-WC) and aqueous layers (F-WA), collected separately, were dried and then tested for antioxidant activity. F-WC which showed antioxidant activity was purified further.

F-WC (33.0 g) was hydrolyzed with 10 mL of 2M HCl at 100 °C for 30 min. The extract so obtained was cooled and filtered and then divided into two portions. First portion of the filtrate was extracted with petroleum ether; ether layer was then separated and evaporated to dryness. The residue dissolved in ether was then separated on silica gel using acetic acid: chloroform (1:9). The major band ($F–WC_{PE}$) obtained in this separation was collected, dried and then analyzed for total phenolics and qualitative nature of the constituent compounds. Second portion of the filtrate was washed twice with ethyl acetate, and ethyl acetate and aqueous layers were collected separately. Aqueous layer was heated at 80 °C for 3 min to remove the last traces of ethyl acetate and the residue, taken in small volume of amyl alcohol, was concentrated to dryness. The dried mass was dissolved in methanolic-HCl and then separated on paper in formic acid: conc. HCl: water (5:2:3) into two major fractions ($F\text{-}WC_{EA1}$ and $F\text{-}WC_{EA2}$). Ethyl acetate layer was divided into two portions: first was chromatographed directly on paper in BAW and the major band ($F\text{-}WC_{EA3}$) was collected while the second was dried, taken up in ethanol, chromatographed on paper in BAW and the major fraction ($F\text{-}WC_{EA4}$) was collected. All the five fractions obtained were tested for their antioxidant activity by DPPH method. Phytochemical screening of the fractions was also carried out in order to identify the major classes of compounds in the fractions. In addition, the antioxidant potential of these was also evaluated in maintaining the stability of the frying oil.

The fractions $L\text{-}WC_{PE}$, $L\text{-}WC_{EA1}$, $L\text{-}WC_{EA2}$, $L\text{-}WC_{EA3}$ and $L\text{-}WC_{EA4}$ were obtained from L-W following the same scheme as for the partition of fraction F-W.

DPPH scavenging activity. Reaction mixture test samples [5 µL dissolved in DMSO (dimethyl sulphoxide) and 95 µL ethanolic solution of 316 µM DPPH (2,2-diphenyl-1-picryl hydrazyl)]in 96-well microtiter plates were incubated at 37 °C for 30 min and absorbance was measured at 515 nm (Yu *et al.,* 2002; Wettasinghe and Shahidi, 2000). Percent inhibition by sample treatment was determined by comparison with a DMSO-treated control group.

Peroxide radical scavenging activity in oil. Ten gram fresh oil were taken in two clean and dry flasks separately (control and test). To one of them, 0.1 g of the test sample was added (test). Both were heated at 98 °C under carefully controlled aerated conditions for 2-3 h. The flasks were then cooled to ambient room temperature and peroxide value of each was determined after cooling (Kochhar and Rossell, 1990; Kahl and Hildebrandt, 1986).

Determination of PV. The test was carried out in diffused daylight. One gram of the oil sample from the control and the test sample each was dissolved separately in 10 mL of chloroform in an iodine flask by stirring. 15 mL of acetic acid and 1 mL of saturated potassium iodide solution were then added, the flasks were stoppered and shaken vigorously for one min and then kept in dark for 5 min. Later, 75 mL distilled water, was added and the liberated iodine was titrated with sodium thiosulphate solution using starch solution as indicator. A blank test was carried out simultaneously without the oil sample under the same conditions (IUPAC, 1987).

Spectroscopy and chromatography. Ultraviolet absorbance, λ_{max} in nm, were measured in methanol, on Shimadzu 160 A UV-visible spectrophotometer. Merck silicagel 60 F_{254} (20 × 20cm) glass plates (5715) were used for analytical thin layer chromatography (TLC).

Phytochemical analysis. Phytochemical screening of the leaf and fruit extracts was undertaken using the methods described by Harborne (1998). The screening covered mainly alkaloids, saponins, flavonoids, tannins and quinones.

Results and Discussion

The results obtained indicated that crude ethanolic extract and aqueous fraction of crude extract produced antioxidative effect (Table 1). This can be explained by the widespread occurrence of polyphenolic compounds,

Table 1. Antioxidant activity of the crude ethanolic extract and aqueous fraction of *T. catappa* fruit and leaf extracts

Extract	DPPH activity (%) Mean ± SD	Peroxide value (meq. O_2/kg) Mean ± SD
Crude ethanolic ext.	80±0.45	2.76±0.06
F-W	75±0.65	3.00±0.13
L-W	78±0.91	2.89±0.09

which are soluble in water, methanol and ethanol. Antioxidant activities of all the fractions derived from leaves were correspondingly higher than those derived from the fruits (Tables 2-3). On comparing the DPPH scavenging activities of the fractions from fruit, the order of activity was found to be F -WC_{PE} > F -WC_{EA4} > F-WC_{EA3} > F-WC_{EAl}= F-WC_{EA2}. The same pattern was observed in the leaf fractions. i.e. L-WC_{PE} > L-WC_{EA4}

Table 2. Antioxidant activity of the *T. catappa* fruit extract

Extract	DPPH activity (%) Mean ± SD	Peroxide value (meq. O_2/kg) Mean ± SD
F-WC_{PE}	68±0.23	3.38±0.09
F-WC_{EA1}	60±0.98	4.50±0.06
F-WC_{EA2}	60±1.2	4.53±0.043
F-WC_{EA3}	62±1.6	4.21±0.21
F-WC_{EA4}	65±0.78	3.56±0.45

Table 3. Antioxidant activity of *T. catappa* leaf extract

Extract	DPPH activity (%) Mean ± SD	Peroxide value (meq. O_2/kg) Mean ± SD
L-WC_{PE}	70±0.45	3.43±0.098
L-WC_{EA1}	60±1.4	4.10±0.056
L-WC_{EA2}	60±2.1	4.10±0.034
L-WC_{EA3}	63±1.6	3.60±0.23
L-WC_{EA4}	68±1.0	3.55±0.25

>L-WC_{EA3} >L-WC_{EAl}= L-WC_{EA2}. This may be due to the relatively simpler structures of the compounds in F-WC_{PE} giving positive test for simple phenols compared to F-WC_{EAl} and F-WC_{EA2}, which gave positive test for anthocyanidins. The fractions also reduced the peroxide formation in the frying oil compared to the control (control peroxide value was 9.01 meq.O_2/kg).

Our results also validate previous reports on the antioxidant activity of the extracts derived from the leaves of *T. catappa* (Chyau *et al.,* 2002; Ko *et al.,* 2002; Wang *et al.,* 2000). Previous phytochemical analysis of fruits and leaves of *T. catappa* revealed the presence of pigments *viz.* violanxanthin, lutein and zeaxanthin and β-cryptoxanthine (Lopez-Hemandez *et al.,* 2001), tannins (Mustapha, 2001; Tanaka *et al.,* 1986; Rayudu and Rajadurai, 1966) and flavone glycosides (Lin *et al.,* 2000). However, when all the bioactive extracts obtained through the scheme adopted in this study were screened chemically, they were found to be simple phenols, anthocyanins, phenylpropanoids and flavonols (Table 4-6). The phytochemical groups of organic compounds detected in these plants have been known to possess antimicrobial properties (Mitscher *et al.,* 1987).

Table 4. Spectral and chromatographic properties of F-W_{PE}

Criterion	Property recorded
Colour	Red-pink Intense green
1% Alcoholic ferric chloride Chromatography: Silica gel, $CHCl_3$: CH_3COOH (9:1)	Best separation
Acid/base response Folin-ciocalteu reagent λ_{max} in HCl-methanol and then in 5% alcoholic $AlCl_3$	Bathochromic shift in alkali Blue colouration 523nm, a bathochromic shift was observed due to catechol group in the molecule

Table 5. Spectral and chromatographic properties of F-W_{EA1} and F-W_{EA2}

Criterion	Property recorded
Colour	Deep red
Two dimension chromatography: BAW and 5% aqueous acetic acid	Best separation
Acid/Base response	Blue/colourless in base and red in acidic medium
λ_{max} in HCl-methanol and then in 5% alcoholic $AlCl_3$	523 nm, a bathochromic shift was observed due to catechol group in the molecule

Table 6. Spectral and chromatographic properties of F-WC_{EA4}

Criterion	Property recorded
Colour	Brown
Colour in UV and UV + ammonia	Bright yellow
chromatography: BAW	Best separation
λ_{max} in 5% alcoholic $AlCl_3$	A bathochromic shift was observed due to catechol group in the molecule

This study supports the concept that *T. catappa* plant may be important in the potential discovery of natural product pharmaceuticals and helps in the scientific validation of the uses of this species as a supplementary support in the suppression of free radical formation which may find one of its applications in controlling/scavenging the free radicals in the frying oil. The free radicals, once initiated in oil, may propagate extensively and produce several other compounds which may lead to rancidity in the oil once or twice for frying, making it completely unfit for further use. The approach of using potential antioxidant in the oil from naturals sources would not only be safe, but can also effectively reduce the cost of frying (Naz *et al.,* 2008; Naz *et al.,* 2005; Naz *et al.,* 2004).

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