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# Performance of Antioxidant Activity of Methanolic Extracts from Different Parts of Some Tree Species using DPPH Radical-scavenging Assay

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**In the present study the tree species namely; *Terminalia arjuna* (Roxb.) (leaves), *Ficus retusa* L. (bark, leaves, wood), *Schinus terebinthifolius* Raddi (fruits), *Ficus benghalensis* L. (fruits), *Ficus carica* L. (fruits), *Cupressus sempervirens* L. (needles), *Delonix regia* (leaves), *Erythrina humeana* (wood, leaves), and *Dalbergia sissoo* Roxb. (wood) were screened and assayed for their antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl method (DPPH). The values of total antioxidant activity (TAA) range from 10±1.02 to 90±2.97%. The highest TAA% was observed by the methanolic extract from *T. arjuna* leaves (90±2.97%), followed by *F. carica* fruits (89±2.7%), *C. sempervirens* needles (88.60±1.51%), *F. benghalensis* fruits (70±3.8%), *F. retusa* leaves (67±1.23%) and *F. retusa* bark. On the other hand, the lowest values were found by the methanolic extract of *F. retusa* wood (10±1.45%) and *D. regia* leaves (13±2.3%) in comparable to Tannic acid as a standard compound (80±2.12%). It can be suggested that the *T. arjuna* (leaves), needles of *C. sempervierins* and fruits of *F. carica* are a great potential source of antioxidant compounds useful for the natural basis.**

**Key words:** Antioxidant activity; Methanolic extracts; DPPH method; Tress.

The free radical scavenging activity and antioxidant activity measured by the 2,2-diphenyl-1-picrylhydrazyl method (DPPH) is described as a simple, rapid and convenient method independent of sample polarity for screening of many samples for radical scavenging activity (Marxen *et al.,* 2007). The method DPPH is widely used for measurement of free radical scavenging ability of antioxidants (Perez-Jimenez and Saura-Calixto, 2008; Perez-Jimenez *et al.,* 2008). For determination of radical scavenging activity of different foods, beverages and substrates were elaborated a great variety of methods with utilization of DPPH (1,1-Diphenyl-2-picrylhydrazyl). They are based on the original methods of Blois (1958) and Brand-Williams *et al.,* (1995). Many authors have been reported the effects of extracts as antimicrobial, antifungal, antioxidant and radical-scavenging properties (Hirasa and Takemasa, 1998) by spices and essential oils and, in some cases, a direct food-related application has been tested (Madsen and Bertelsen, 1998). That means that the comparison between the values reported by different laboratories can be quite difficult (Pérez-Jiménez *et al.,* 2008). The use of plants for their therapeutic value is a part of the human history in Egypt (Ali *et al.,* 2012). Natural products derived from higher plants have received considerable attention in

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recent years due to their diverse pharmacological properties (Govind and Sahni, 2011). Herbal drugs containing antiradical constituents are gaining importance in prevention and treatment of oxidative stress linked diseases (Anand and Shrihari, 2011).

The goal of this investigation is to measure and evaluate the free radical scavenging activity of methanolic crude extracts from different parts of some trees by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method DPPH and comparing these activities with the value from Tannic acid as a standard antioxidant activity.

## MATERIALS AND METHODS

### Plant material

The different parts of the following tree materials were collected in the period of 2012-2013 from different location at Alexandria city, Egypt; *Terminalia arjuna* (Roxb.) (leaves), *Ficus retusa* L. (bark, leaves, wood), *Schinus terebinthifolius* Raddi (fruits), *Ficus benghalensis*

L. (fruits), *Ficus carica* L. (fruits), and *Cupressus sempervirens* L. (needles) and were kindly identified at the department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University. The tree species (*Delonix regia* (leaves), *Erythrina humeana* (wood, leaves), and *Dalbergia sissoo* Roxb. (wood) which are growing at Al-Diriyah city located on the northwestern outskirts of the Saudi capital, Riyadh, Saudi Arabia, were provided by the laboratory of Botany and Microbiology Department, College of Science, King Saud University.

### Extraction of plant material

The bark, wood, fruits and leaves materials were shade air-dried at room temperature for one week and pulverized to a powder using a laboratory small mill. About 30 g of the air-dried parts from the tree species were macerated in 80% methanol (200 ml) for one week under room temperature (Ali *et al.*, 2013). The extracts were filtered, concentrated, lyophilized and stored in a dark vials at 4°C for further use. The crude extract was dried and weighted.

### DPPH radical-scavenging assay

Free radical scavenging activity of the methanolic crude extracts from different parts of the selected trees was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Elansary *et al.*, 2012) with some modifications. An aliquot of 2 mL of stock solution of 0.1 mM DPPH (Sigma-Aldrich) reagent dissolved in pure methanol was added to a test tube with 2 mL of the sample solution in methanol (200 µg/L). The reaction mixture was mixed for 10 s and left to stand in fibber box at room temperature in the dark for 30 min. The absorbance was measured at 517 nm, using a UV scanning spectrophotometer (Unico® 1200). Pure methanol (Sigma-Aldrich) was used to calibrate the spectrophotometer. The decrease in the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. Total antioxidant activity (TAA %) was expressed as the percentage inhibition of the DPPH radical and was determined by the following equation; TAA (%) = $(A_0–A_s/A_0)\times100$; where TAA is the total antioxidant activity, $A_0$ is the absorbance of DPPH solution in methanol and $A_s$ is the absorbance of a DPPH solution with a tested fraction solution (test) or Gallic acid (positive control) solution. The control contained 2 mL of DPPH solution and 2 mL of methanol. It should be noted here that the TAA% value was taken from the previous work (Abdel-Megeed *et al.*, 2013).

### Statistical analysis

The average values of total antioxidant activity (TAA %) were carried out for three replicates. The results are expressed as mean values ± standard deviation (SD).

## RESULTS AND DISCUSSION

The quantities of methanloic extracts of different parts from the trees under this study are presented in Table 1. The quantities range from 4.5% (*E. humeana* wood) to 24.34% (*E. humeana* bark).

The highest TAA% was observed by the methanolic extract from *T. arjuna* leaves (90±2.97%), followed by *F. carica* fruits (89±2.7%), *C. sempervirens* needles (88.60±1.51%), *F. benghalensis* fruits (70±3.8%), *F. retusa* leaves (67±1.23%) and *F. retusa* bark. On the other hand, the lowest values were found by the methanolic extract of *F. retusa* wood (10±1.45%) and *D. regia* leaves (13±2.3%).

The stem bark of *T. arjuna* (Roxb.) is used by the Ayurvedic physicians in India for the treatment of various cardiovascular diseases,

collectively referred to as hritroga Maulik and Katiyar (2010). Various bioactive compounds, like triterpinoids, tannins, flavonoids and minerals have been isolated from the stem bark (Maulik and Katiyar, 2010). Its stem bark possesses glycosides, large quantities of flavonoids, tannins and minerals. Flavonoids have been detected to exert antioxidant, anti-inflammatory and lipid lowering effects while glycosides are cardiotonic, thus making *T. arjuna* unique amongst currently used medicinal plants (Oberoi *et al.,* 2011; Dwivedi 2007).

Methanolic extract of *S. terebinthifolius* fruits exhibited the TAA% of 50±2.34% and the leaves had the value of 55±2.89% and this value considered to be a good as antioxidant activity. Bendaoud *et al.,* (2010) reported that the essential oils from leaves of *S. terebinthifolius* had the great antioxidant activity**.** It seems to be a general trend that the essential oils which contain monoterpene hydrocarbons, oxygenated monoterpenes and/or sesquiterpenes have greater antioxidative properties (Tepe *et al.,* 2004; Mau *et al.,* 2003). It

**Table 1.** The metanolic crude extracts from differentparts of studied tree species and their antioxidant activities

| Tree | Part used | MeOH quantity [a] | TAA% [b] | TA% [c] |
|---|---|---|---|---|
| *T. arjuna* | leaves | 8.8% | 90±2.97% | (90±5.12)% |
| *F. retusa* | bark | 20% | 60±1.02% | |
| | leaves | 13.90% | 67±1.23% | |
| | wood | 5% | 10±1.45% | |
| *S. terebinthifolius* | fruits | 15.9% | 50±2.34% | |
| | leaves | 14.7% | 55±2.89% | |
| *F. benghalensis* | fruits | 15.7% | 70±3.8% | |
| *F. carica* | fruits | 18.90% | 89±2.7% | |
| *C. sempervirens* | needles | 21.34% | 88.60±1.51% | |
| *D. regia* | leaves | 10.4% | 13±2.3% | |
| *E. humeana* | leaves | 12.8% | 32±1.34% | |
| | wood | 4.5% | 15±1.98 | |
| | bark | 23.12% | 40±3.13% | |
| *D. sissoo* | wood | 12.98% | 40±2.56% | |

a: based on oven dry weight b: TAA%; Total antioxidant
c: TA%; Tannic acid (Abdel-Megeed *et al.,* 2013)

appears that the observed radical scavenging properties of *S. terebinthifolius* essential oil might contribute positive effects in the defense of *S. terebinthifolius* (Gundidza *et al.,* 2009). *S. terebinthifolius* produce resin-like materials which contain monoterpenes to defend themselves against the penetrations of the attacking pathogens (Byers, 1995). Tannins, saponins and polyphenol *S. terebinthifolius* could be the major components that inhibited the growth of Jurkat cells (Woraratphoka *et al.,* 2012; Queires et al., 2006).

The TAA% found in methanolic extracts of wood, leaves and bark of *F. retusa* were 10±1.45%, 67±1.23% and 60±1.02%, respectively. The antioxidant strongest activities of *F. microcarpa* (*F. retusa*) bark and leaves extract may be attributed to its high level of phenolic compounds like flavonoids, coumarin and triterpenoids (Ao *et al.,* 2008). On the other hand, the plant showed a moderate antioxidant activity and the ethyl acetate and n-butanol fractions had owned the high activity (Abdel-Hameed, 2009). Additionally, the golden yellow leaves was found to have a high amounts of flavonoids, carotenoids, triterpenoids, fatty alcohol, steroids, coumarins, flavane-4-hydroxybenzoate and isoflavones (Chiang and Kuo, 2003; Takahashi *et al.,* 2002; Li *et al.,* 1998). The main compounds presence in the ethyl acetate fraction from the leaves of *F. retusa*, were 1,2-benzenedicarboxylic acid-dibutyl ester, phenol,4-(2-aminopropyl)-, and R-(2,2,3,3-2H4) butyrolactone (Aly *et al.,* 2013).

The methanolic extract of fruits of *F. benghalensis* had a strong antioxidant activity (70±3.8%). The previously phytochemical screenings of *F. benghalensis* revealed the

presence of saponins, tannins and flavonoids in aqueous and methanolic extract (Manimozhi *et al.,* 2012). The methanolic crude extract contains glycoside; 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitosterol-alpha-D-glucose and meso-inositol have been isolated from the bark (Mousa, 1994).

Methanolic extract of *F. carica* fruits had a great TAA% (89±2.7%). The higher the polyphenol content (anthocyanins), polysaccharides, flavonoid compounds, phytosterols, and fatty acids, sugars and mineral salts in *F. carica* fruit, was correlated the higher antioxidant activity (Caliskan and Polat, 2011; Yang *et al.,* 2009; Veberic *et al.,* 2008; Aljane *et al.,* 2007; Solomon *et al.,* 2006; Jeong and Lachance, 2001). The main phenolic compounds in *F. carica* were cyaniding 3-rutinoside, cyanidin 3-glucoside, cyanidin 3,5-diglucoside and pelargonidin 3-rutinoside (Solomon *et al.,* 2006), hydroxycinnamic acid derivatives, such as 3-O- or 5-O-caffeoylquinic acids and ferulic acid; flavonoid glycosides such as quercetin 3-O-glucoside and quercetin 3-O-rutinoside; and furanocoumarins such as psoralen and bergapten (Del Caro and Piga, 2008; Oliveira *et al.,* 2009).

The methanolic extract of needles from *C. sempervirens* had a great TAA% 88.60±1.51%. Previously, the methanolic extracts of *C. sempervirens*, showed a remarkable radical scavenging effect at low concentrations (Mothana *et al.,* 2009). The essential oils were showed a good antioxidant activity (Elansary *et al.,* 2012; Sacchetti *et al.,* 2005). The phenolic compounds extracted from leaves of *C. sempervirens* grown in Egypt showed high antioxidant activity (Ibrahim *et al.,* 2007). The hydroethanolic extract of leaves of *C. sempervirens* was showed good antioxidant activity comparing with ascorbic acid (Ali *et al.,* 2012). Previously the extracts from *C. sempervirens* is rich in flavonoids (cupressuflavone, amenoflavone, rutin, quercitrin, quercetin, myricitrin) and phenolic compounds (anthocyanidin, catechines flavones, flavonols andisoflavones) tannins, catchol and essential oil which have a great antioxidant activity (Koriem, 2009; Mazari *et al.,* 2010).

In the present study the TAA% of *D. regia* leaves was 13±23% and this seems to be very low in comparable with the value obtained from TA (90±5.12). Previously, the TAA% of bark extract was 78.35±1.45 % and 73.40±2.67 % and these values are close to the TAA % showed by Gallic acid (80±2.12 %) (Salem, 2013). In *D. regia*, the mostly secondary metabolites such as flavonoids and sterols (Guerere *et al.,* 1986; EL-Sherbeing *et al.,* 1971; Subramanian *et al.,* 1966) tannins and phenol compounds were found. Some carotenoids like 2-carotene, zeaxanthein etc. (Jungalwala and Cama, 1962) also exhibits antimicrobial activities. Gallic acid in in the bark as the main phenolic compound was found and other phenolic acids such as sorbic, sinapic, p-coumaric, m-coumaric, ferulic, caffeic, 3-hydroxybenzoic, 4-hydroxycinnamic and 4-hydroxybenzoic acids were also presented (Shabir *et al.,* 2011).

Methanolic extract of wood and leaves from *E. humeana* showed a weakly antioxidant activity (TAA of 15±1.98 and 32±1.34%, respectively). Previously, Salem (2013) reported that stem bark methanolic extract had a value of 73.40±2.67 % and these value was closed to the TAA % showed by Gallic acid (80±2.12 %). Phytochemical analysis has revealed the presence of terpenes in plants from the *Erythrina* genus (Nkengfack *et al.,* 1994). The bioactive alkaloid-rich plants and flavonoids, especially, isoflavones, pterocarpanes, flavanones and isoflavanones were also reported (Chacha *et al.,* 2005; Barakat *et al.,* 1977).

High phenolic compounds in methanol extracts play the role of these activities and possess potent antioxidants and antimicrobial. Flavonoids have been proven to display a wide range of pharmacological and biochemical actions, such as antimicrobial and anticarcinogenic activities (Cook and Samman, 1996). Flavonoids can act as free radical scavengers and terminate the radical chain reactions that occur during the oxidation of triglycerides in the food systems (Roedig-Penman and Gordon, 1998). Moreover, the presence of tannins in the extracts may explain its potent bioactivities are known to possess potent antioxidants (Zhang and Lin, 2008).

*D. sissoo* Roxb. belongs to family: Fabaceae is commonly known as Indian rosewood (Kumar and Kumud, 2010). The TAA% of wood found to be 50±2.34%. The extract was found to possess antidysentric, analgesics and antipyretic (Brijesh *et al.,* 2006; Hajare *et al.,* 2000). The bark

and extracts of wood are bitter used as aphrodisiac, abortifacient, expectorant, antihelmintic and antipyretic (Upwar *et al.,* 2011). The phytochemical analysis of *D. sissoo* reported the presence of alkaloids, carbohydrates, saponins, flavonoids, glycosides and steroids (Reddy *et al.,* 2008). The extracts from stem bark have been reported to have a good antioxidant activity (Roy *et al.,* 2011).

## CONCLUSION

In the present study diifernt parts from some tree species grown under the Egyptian condition were screened for their antioxidant activity using DPPH method. The results showed that the highest TAA% was observed by the methanolic extract from *T. arjuna* leaves (90±2.97%), followed by *F. carica* fruits (89±2.7%), *C. sempervirens* needles (88.60±1.51%), *F. benghalensis* fruits (70±3.8%), *F. retusa* leaves (67±1.23%) and *F. retusa* bark. On the other hand, the lowest values were found by the methanolic extract of *F. retusa* wood (10±1.45%) and *D. regia* leaves (13±2.3%) in comparable to Tannic acid as a standard compound (80±2.12%). %). It can be suggested that the *T. arjuna*, *C. sempervierins* and *F. carica* are a great potential source of antioxidant compounds useful for the natural basis.

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