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In vitro evaluation of antioxidant activity of *Caryota mitis* Lour. Leaves extracts

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Abstract
Free radicles surly attack different tissues of the body causing serious cell damage and various inflammatory conditions. As a result, there is an urgent need for discovery of new and safe antioxidants of natural sources. DPPH• radicle scavenging assay is an accurate and precise spectrophotometric method used for determination of antioxidant activity, this depends on the measurement of degree of DPPH• color change from violet to yellow spectrophoto metrically. When this technique was applied on the total and different extracts in addition to two pure flavonoids isolated from the leaves of *Caryota mitis* Lour., the result revealed that the plant exhibited strong antioxidant activity relative to that of standard ascorbic acid which open the field for industrial formulation of new and safe antioxidant product.

Keywords: Antioxidant, DPPH, *Caryota mitis*, flavonoids

Introduction
Free radicals (FRs) are found in nature in a wide range of biological and chemical systems [1]. They are chemical stable atoms and molecules, which possess usually one somtimes more free electron/electrons in the electron envelope [2]. Almost all biomolecules, but mainly biomembranes, proteins and nucleic acids, may be attacked by reactive free radicals [3, 4]. Human cellular damage may be a result of these free radicals attack causing numerous diseases including inflammatory disorders, atherosclerosis and cancer [5]. The protective system of the organisms against these FRs is based on the activity of specific enzymes (especially superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase) as well as nonenzymatic compounds with antioxidant activity (α-tocopherol, L-ascorbic acid, glutathione, coenzyme Q10, flavonoids [6]. By reviewing the literature, genus *Caryota* exhibited analgesic, antirheumatic, antimicrobial and antioxidant activities [7-10], and there is no reports about antioxidant activity of *Caryota mitis*. So, in this study the main aim is to evaluate the antioxidant activity of different extracts in addition to two pure flavonoids (quercetin-3-O-β-D-glucoside and rutin) isolated from *Caryota mitis* using DPPH• radicle scavenging assay.

Equipment
UV spectra were recorded in methanol on Ultrospec 1000, UV-VIS spectrometer, Pharmacia Biotech, Cambridge (Cuvette of 1cm x 1cm x 4.5 cm). Rotary Evaporator 4000 (Heidolph, Germany).

Plant material
Fresh samples of *C. mitis* leaves were collected during the flowering stage in June 2013 from El-Orman Botanical Garden, Giza, Egypt. The plants were kindly identified by Mrs. Traes Labib, general manager of plant taxonomy in El-Orman Botanical Garden, Giza, Egypt.

Chemicals
2, 2-Diphenyl-1-picryl hydrazyl (DPPH•) was purchased from Sigma-Aldrich Chemicals Co., Germany. Ascorbic acid was obtained from El-Nasr Pharmaceutical and Chemical Co., Egypt (ADWIC). Other chemicals used were of high analytical grade and obtained from Merck Chemical Co., Germany.

Preparation of plant extracts
Five kilograms (5 Kg) of the air dried powdered leaves were extracted by maceration with 70 % ethanol at room temperature till exhaustion. The extract was concentrated under reduced pressure and left to dry to obtain solid residue (465 g). This residue was suspended in 500 ml water and subjected to solvent fractionation using n-hexane, dichloromethane, ethyl acetate and n-butanol respectively.

Each fraction was concentrated under reduced pressure and left to dry then weighed to give 195, 3.5, 32, 15 g respectively, leaving remaining aqueous fraction of 130 g.

DPPH· Radical Scavenging Activity (DPPH· assay)

Antioxidant activity was determined by the DPPH· method [11], based on the reduction of alcoholic DPPH· solutions at 517 nm in the presence of a hydrogen donating antioxidant (AH) due to the formation of the non-radical from DPPH-H by the reaction:

$$DPPH^{\cdot} + AH \longrightarrow DPPH\text{-}H + A$$

The concentration of DPPH· was kept at 100 µM in MeOH. The radical scavenging activity was measured by spectrophotometric method, in which 0.2 ml of methanolic solutions of n-hexane, chloroform, ethyl acetate, n-butanol and aqueous extracts (62.5, 125, 250, 500 and 1000 µg/ml) in addition to two pure flavonoids quercetin-3-O-β-D-glucoside and rutin in the same concentrations were mixed with 2 ml of methanolic solution of DPPH· (100 µM). Similarly, 0.2 ml of methanolic solutions of ascorbic acid (62.5, 125 250, 500 and 1000 µg/ml) were added to 2 ml DPPH· and used as a positive control. A mixture of 0.2 ml of methanol and 2 ml of methanolic solution of DPPH· (100 µM) served as control. After mixing, all the solutions were incubated in dark for 30 minutes and the absorbance was measured at 517 nm. The experiments were performed in triplicate and percent scavenging activity was calculated as follows:

$$\text{Scavenging \%} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Results

The listed results in (Tables 1 & 2), revealed that the ethyl acetate extract, quercetin-3-O-glucoside and rutin exhibited strong antioxidant activity (Fig. 1), and high relative activity in comparison with ascorbic acid (Fig. 2). The total methanolic extract in addition to chloroform and n-butanol fractions showed moderate antioxidant activity, while the n-hexane and aqueous fractions showed the weakest free radical scavenging activity.

Table 1: Antioxidant activity percent (%) of the total methanolic extract, different fractions and pure compounds of *C. mitis* Lour. leaves.

Extracts / Concentration	Antioxidant (%)				
	1 mg/ml	**0.5 mg/ml**	**0.25 mg/ml**	**125 µg/ml**	**62.5 µg/ml**
DPPH (Blank)	-	-	-	-	-
Ascorbic acid (reference)	91.9	91	90.8	85.8	67.4
Total MeOH extract	83.2	49.4	28.7	15.6	9.9
n-Hexane fr.	14.1	8.1	4.2	1.3	0.8
Chloroform fr.	82.4	57.8	35.5	17.9	10.9
Ethyl acetate fr.	92.4	92.2	90.4	62.4	33.6
n-Butanol fr.	88.5	59.3	34.8	32.1	18.3
Aqueous fr.	35.1	17.1	9.9	4.8	2.5
Quercetin-3-O-glucoside	86.44	86.2	85.3	85	48.1
Rutin	83.7	83.2	82.3	80.8	52.1

Table 2: Relative antioxidant activity of the total methanolic extract, different fractions and pure compounds of *C. mitis* Lour. in comparison with ascorbic acid (as reference).

Extracts / Concentration	(%) Relative antioxidant activity				
	1 mg/ml	**0.5 mg/ml**	**0.25 mg/ml**	**125 µg/ml**	**62.5 µg/ml**
Total MeOH extract	90.5	54.3	31.6	18.2	14.7
n-Hexane fr.	15.3	8.9	4.6	1.5	1.2
Chloroform fr.	89.7	63.5	39.1	20.9	16.1
Ethyl acetate fr.	100.5	101.3	99.6	72.7	49.9
n-Butanol fr.	96.3	65.2	38.3	37.4	27.2
Aqueous fr.	38.2	18.8	10.9	5.6	3.7
Quercetin-3-O-glucoside	94.1	94	93.9	99.1	71.4
Rutin	91.1	91.4	90.6	94.2	77.3

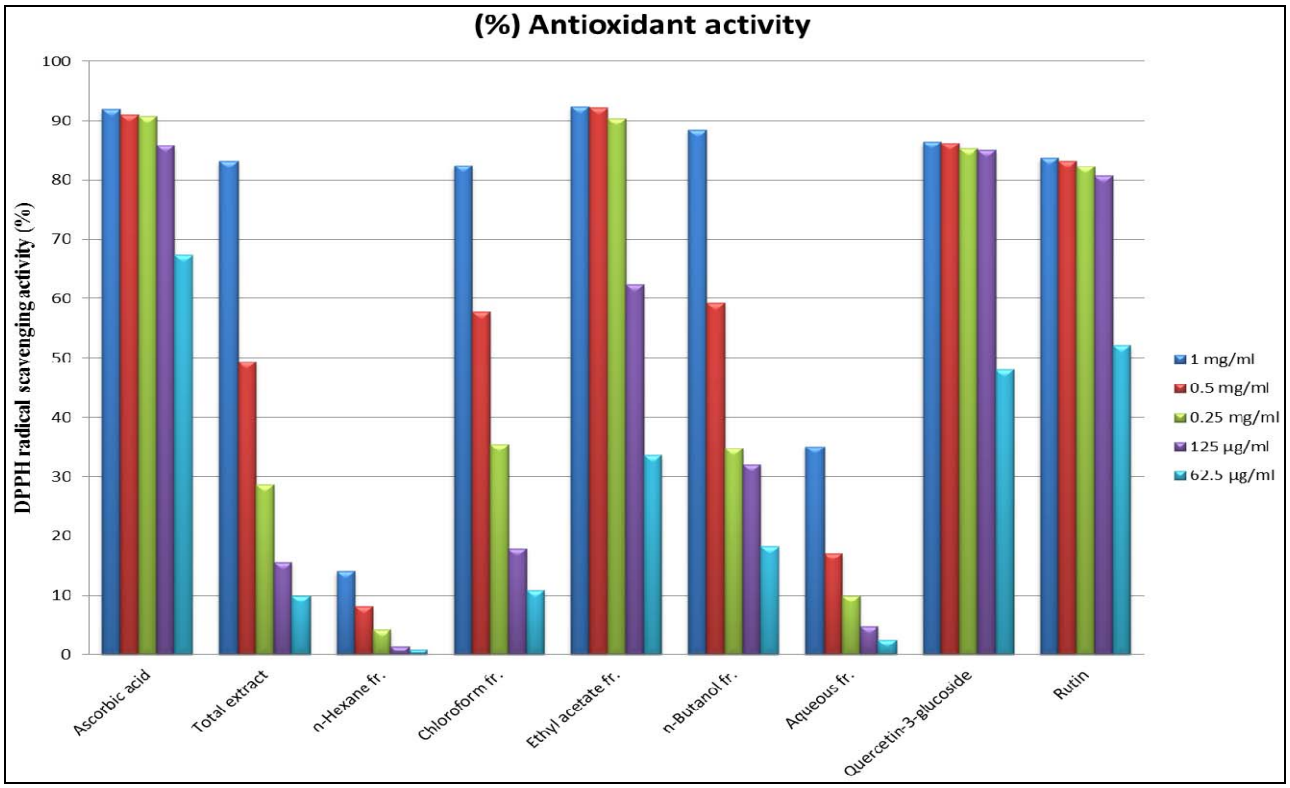

Fig 1: DPPH radical scavenging activity of the different extracts of the leaves of *C. mitis*

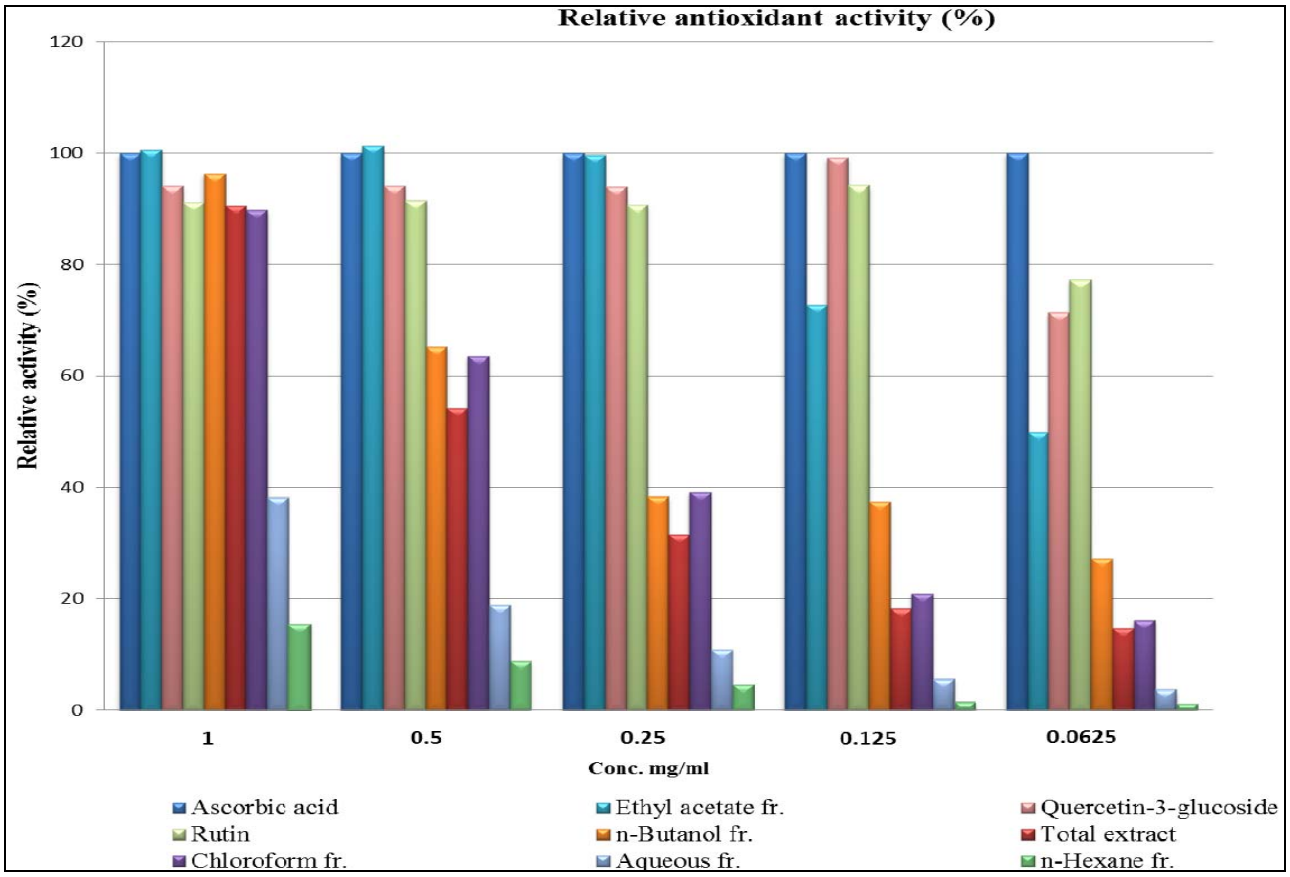

Fig 2: DPPH• radical scavenging activity of the different extracts of the leaves of *C. mitis*

Discussion

The ethyl acetate fraction displayed the strongest free radicle scavenging activity and this is attributed to the high proportion of flavonoids and phenolic acids, which have the ability to easily reduce free radicals [12, 13]. Furthermore, quercetin-3-O-glucoside and rutin (flavonols glycosides) exhibited promising antioxidant activity, which lasts even at concentration of 62.5 µg/ml and this is probably due to the

high reducing capacity of this class of compounds that is acquired by the presence of numerous free hydroxyl groups in addition to the double bond between C_2-C_3 in conjugation with 4-oxo group that are involved in radical stabilization via electron delocalization over the three ring system [14].

Conclusion

Antioxidant activity assay using DPPH• model was performed on the different extracts of the leaves of *C. mitis* as well as two pure isolated flavonoid glycosides from the plant. The results revealed that *C. mitis* extracts and fractions have very powerful antioxidant activity in comparison with ascorbic acid as a reference standard, especially the ethyl acetate fraction and the two flavonoids quercetin-3-O-β-D-glucoside and rutin.

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