

In Vitro Antioxidant Activity of Ethanolic Extract from *Terminalia Catappa* (L.) Leaves and Fruits: Effect of Fruit Ripening

Abdulaziz Rabi Abdulkadir^{1*}

^{1*}Faculty of Bioresources and Food Industry, Universiti Sultan Zainul Abidin, Tembilaka campus Besut, Terengganu, Malaysia

***Corresponding Author:**

Abdulaziz Rabi Abdulkadir¹

Faculty of Bioresources and Food Industry, Universiti Sultan Zainul Abidin, (UNISZA)
Tembilaka campus Besut, Terengganu, Malaysia
Tel: +601126982159, Email: abdulwarure@yahoo.com

Abstract: *Terminalia catappa* L. popularly known as tropical almonds, sea almonds and Indian almonds. It is widely distributed to many tropical countries ranging from India, Malaysia, Australia, and Pakistan among others. It has been used traditionally to cure diseases like dysentery, bilious fever, diarrhoea, sores and abscesses. The aim of this study was to evaluate antioxidant activity of leaf and fruit of *Terminalia catappa*, with regard to fruit ripening. Total phenolic and total flavonoid content of the samples were assessed using the *in vitro* Folin–Ciocalteu method and aluminium chloride assay respectively. While antioxidant potential of the samples were evaluated via DPPH radical scavenging and Ferric-reducing antioxidant power assays. Leaf extract showed the highest levels of total phenolic, total flavonoid content, DPPH radical scavenging activity and reducing power potential, with 285.77 ± 4.83 (mg GAE/ g), 59.95 ± 3.41 (mg QAE/ g), 43.34 IC_{50} ($\mu\text{g/ml}$) and 2512.89 ± 13.47 (mM Fe (II)/g) respectively. However, nearest all the activities studied increases as the ripening progressed. Thus ripe fruit showed highest levels of total phenolic, total flavonoid content, DPPH radical scavenging activity and reducing power potential compared to unripe fruit. This may be related to the increased of vitamin C content through ripening process.

Keywords: DPPH antioxidant, FRAP assay, phenolics, flavonoids, ripening

1. Introduction

Terminalia catappa refer to as Tropical almond, it is large tree that grow in tropical countries with a maximum height of 35 m, being upright, with horizontal branches and symmetrical crown [1]. Its trunk is 1-1.5 m in diameter, frequently buttressed at the base, the leaves were obovate and alternate between one another, petioles are short, spirally gathered at the branch tips, the leaves are dark green above, paler underneath, glossy and or leathery. Sometimes change to bright scarlet, yellow, dark red and or dark purple. the fruit appeared rounded and flattened, some times egg like shaped, with a green or red in colour and occasionally changed to radish or yellow after ripened [2]. [3] Observed phenotypic variation of *T. catappa* grown in south-western Nigeria, thus changes in fruit colour leaf shape were observed, across the studied area. It is native to Australia, Malaysia, India, Thailand, Cambodia, Laos, Japan, Vietnam and non native to Philippines, Afghanistan, Bangladesh, USA, Netherlands, Brazil, Colombia, Singapore, Pakistan. The plant is quite distributed across the globe, thus different native called it in a different way, example Arabic (Brasilia); Malay (ketapang); Bengali (bangla-badam); Dutch (amandel boom); French (amandier de la Martinique,); English (sea almond, tropical almond, Indian almond-wood); Portuguese (parasol, amendoeira); German (Katappenbaum); Hindi (badambo,); Spanish (almendron); Swahili (mkungu); Thai (taa-pang) [2]. It is reported that leaf, bark and fruit of *Terminalia catappa* is a

rich source of allagic acid, corilagin, gallic acid and many more unidentified flavonoids compound [4]. The bark is excellent source of tannins [1]. However, some phenolcarboxylic acids, and two phenol glucoside gallates, tannin, catappanin A, hydrolyzable tannin, seven ellagic tannins, four flavan-3-ols, two complex type tannins were reported present in the bark of *Terminalia catappa* [5]. Owing to the above mentioned chemical compounds, the leaves, stem, fruits and bark were used traditionally to cure many diseases, example, leaves and fruit, are astringent, the leaves are combine with rhizomes of *Cyperus rotundus* and *Dacrydium elatum* to treat dysentery, leaves, fruits and bark were used to cure yaws while root and bark serve as a treatment for bilious fever, thrush, diarrhoea, and to cure sores and abscesses, the fruits kernel has been used with beeswax as a remedy for bloody faeces and to stop putrid exudation [2]. Nevertheless, juices from the leaves were used as an ointment for scabies, leprosy and other skin infections [4].

Recently many researchers revealed pharmacological properties of the plant, among which are anti-diabetic [6], anti-inflammatory [7], anti-hepatitis [9], antibacterial [10] and many more.

Antioxidants are chemical compounds that can hinder damages caused by free radicals in the cells, which might eventually causes many diseases [11]. [12] Described free radicals as extremely reactive compounds that can easily react with DNA and many macromolecules present in the cells and ultimately cause injury to the cell. Although,

mechanism action of antioxidant compounds is subjected to its ability to scavenge free radicals via donation of electron or hydrogen. Phenolic compounds are important component of plant based foods that provide the attractive colour of juices, red fruits and wines as well as flavour of many plants [13]. However antioxidant activities of most fruits plant majorly relied on phenolics (e.g. phenolic acid, flavonoids), vitamins, carotenoids and glutathione [14]. [15] Discovered that antioxidant, total phenolic content and anthocyanin increases with increase in fruit maturity, thus a ripe fruits may have high antioxidant activity than the unripe one. The aim of this study is to evaluate antioxidant activity of leaf and fruit of *Terminalia catappa*, and to study the effect of fruit ripening on antioxidant activity.

2. Material and Methods

2.1 Chemicals

1, 1-diphenyl 2-picrylhydrazyl radical (DPPH), Folin-Ciocalteu, quercetin, Dimethylsulphoxide (DMSO), Na_2CO_3 , tripyridyltriazine and ethanol were used in this experiment, percentage of antioxidant activity of the samples used were determined via 1, 1-diphenyl 2-picrylhydrazyl radicals (DPPH) and Ferric-reducing antioxidant power assays (FRAP). Which were measured, according to a standard technique of measuring antioxidant activity.

2.2 Plant Material

Fresh leaves and fruits of *Terminalia catappa* L. were collected from universiti Sultan Zainal Abidin, Gongbadk campus, Terengganu, Malaysia. The plants were authenticated by the Faculty of Bioresources and Food Industry, Universiti Sultan Zainul Abidin, (UNISZA). Tembila campus, Terengganu, Malaysia. The plant was preserved at the university herbarium.

2.3 Extract preparation

The plant samples were washed properly and separated into leaf, ripe fruit (yellow) and unripe fruit (green). Followed by subsequent dried at $40-43^\circ\text{C}$. The dried samples were extracted using 95% ethanol. The mixture were filtered through Whatman number 1 filter paper and concentrated on rotary evaporator (EYELA N-1110, Tokyo) at $45-50^\circ\text{C}$, it is then dried and kept at 4°C prior to use for the assay. The solvent was two times mass of the sample during extraction. The extracts were dissolved in DMSO, or methanol to get the final concentration as per requirement [16].

2.4 Total phenolic content assay (TPC)

The total phenolic content of the extract was determined by adopting modified method of [17] with some additional modification. Folin-Ciocalteu (F-C) reagent was used in the test, 250 μL of extract diluted in DMSO was put in a test tube and subsequent mixed with 1.25ml of F-C reagent diluted in distilled water 1:9, and incubated for 10 minute, 1ml of 7.5% Na_2CO_3 solution was then added, which was further incubated for 30minute in dark prior to measurement at 650nm using spectrophotometer. Garlic acid was used as a standard.

2.5 Total flavonoid content assay (TFC)

The total flavonoid content was determined using a method adapted from [18] with some few modification. Aluminium chloride (AlCl_3) mixture and quercetin standard was used to make the calibration curve. 0.32mg/ml of quercetin was used for the experiment; it was further diluted to various concentrations. A calibration curve was made through measuring the absorbance at 415nm from each dilution using a spectrophotometer. Aluminium chloride, 10% and 1M potassium acetate solutions were used. When aluminium chloride react with compounds having flavonoids groups, it usually forms an acid stable complexes via the C-4 keto group or between C-3 and C-5 OH group, nevertheless, aluminium chloride forms acid labile complexes through the ortho - dihydroxyl groups at a position of A- or B-ring of the flavonoids.

2.6 1, 1-diphenyl 2-picrylhydrazyl radical assay (DPPH)

Antioxidant activity of extracts from different leaf and fruit of *Terminalia catappa* on DPPH was tasted using the method of [19] with some modification. 500 μL of the extract diluted in methanol was mixed with 1ml of DPPH in methanol (0.3mM) in test tubes. The tubes were kept in the dark for about 30 min, and the absorbance of the solution was measured at 517 nm using UV-VIS spectrophotometer (SHIMAZU). A blanks containing methanol only, were run with quercetin solutions dissolved in methanol which served as a standard. Extracts were first tested at a single concentration of 0.3mM, followed by subsequent serial dilution which resulted to a range of concentrations through which IC_{50} was established (the concentration reducing DPPH absorbance by 50%).

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{[A_0 - A_1]}{A_0} \times 100$$

Where, A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the methanolic plant extract.

2.7 Ferric-reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to the modified method of [20] with a slightly modification. The FRAP reagent was prepared by combining 10 mmol TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mmol HCl, 20 mmol iron(III) chloride aqueous solution and acetate buffer (pH 3.6) in the ratio 1:1: 10 (v/v), respectively. FRAP reagent was prepared fresh for the experiment and was warmed to 37°C in a water bath for 30 minutes prior to use. Fifty microliters of sample were added to each 1.5 mL of the FRAP reagent. The absorbance of the reaction mixture was measured at 593 nm with UV-VIS spectrophotometer (SHIMAZU), after 30 minutes of incubation. 2000 μM of the iron (II) sulfate solution was used as a standard and further diluted to 1000, 500, 250, 125, 62.5 and 31.25 μM , and the results were converted to mmol Fe(II)/g dry weight of plant material. All of the measurements were taken in triplicate and the mean values were calculated.

2.8 Statistical Analysis

Each assay was subjected to one way analysis of variance using statistical Package for Social Sciences (SPSS).

Significance level of 0.05 % was used to test differences between the samples used.

3. Result and Discussion

3.1 Result

3.1.1 Total phenolic and total flavonoid content

The result of total phenolic content and total flavonoid content of the samples were calculated using the equation

obtained from the standard curve of gallic acid and quacetin acid respectively (Table 1). However, the result were interpreted in terms of GAE and QAE of the extract respectively,

Where Y is the average absorbance of the sample while X is the amount of galic acid or quacetin acid in µg/ml.

Table 1: linear equations and their R² values obtained from the standard calibration curve

Assays	Calibrations curve	R ²
TFC	Y= 0.0025X+0.0708	0.9986
TPC	Y= 0.0088X+0.0932	0.9991
FRAP	Y= 0.0003X+0.7706	0.9995

The results in Table 2 revealed significant phenolic content from all samples, the total phenolic content from leaf extract was 285.7727 (mg GAE/ g) and it significantly differ with that of fruit extract at (P < 0.05). However extract from ripe fruit was found to contain higher phenolic content 117.0985 (mg GAE/ g) which significantly differ with that of unripe fruit with 76.3788

(mg GAE/ g). Nevertheless, flavonoid content also follow the same trends as that of phenolic content, where total flavonoid of the three samples varied significantly at (P < 0.05), leaf extract revealed higher value with 59.9467(mg QAE/ g), followed by ripe fruit 28.2133 (mg QAE/ g) and finally unripe fruit extract 6.7467 (mg QAE/ g) (Table 2).

Table 2: Estimation of total phenolic and total flavonoid content from leaf and fruit of *Terminalia cattapa* with respect to fruit ripening

Sample	TPC (mg GAE/ g)	TFC (mg QAE/ g)
Leaf	285.77 ± 4.83a	59.95 ± 3.41a
Ripe fruit	117.10 ± 3.67b	28.21 ± 0.83b
Unripe fruit	76.3788 ± 2.24c	6.7467 ± 0.23c

a, b & c Indicates a significant Different at (P < 0.05)

3.1.2 DPPH antioxidant assay

DPPH radical scavenging assay majorly involve in scavenging of hydrogen radicals, which seem to have a characteristic absorption of 517 nm, particularly when it scavenge the proton-radical, which rapidly change the original colour of DPPH solution in to yellow. Antioxidant activity of the extract from leaf and fruit of *Terminalia cattapa* were studied and the results were compared with that of the control. The result revealed significant difference between all samples used as the (P < 0.05). Percentage inhibition of unripe fruit is significantly higher than that of ripe fruit, control and leaf extract, however, there is no significant difference between control and ripe

fruit, while significant difference was observed between control and leaf extract. Highest percentage inhibition was recorded from unripe fruit with 90.4929 (%), followed by ripe fruit 89.4586 (%), control 89.2846 (%), and finally leaf extract 88.9777 (%). The real activities of the samples were compared by the amount of antioxidant required to scavenge 50% of DPPH free radicals (IC₅₀) (Table 3). The lower the IC₅₀ signifies the higher antioxidant activity. The result shows that leaf and ripe fruit extract having lowest IC₅₀ 43.34 (µg/ml) and 95.99 (µg/ml) respectively, compare to control with IC₅₀ 107.61(µg/ml) and unripe fruit with IC₅₀ 211.06 (µg/ml) (Table 3).

Table 3: DPPH antioxidant activity of ethanolic extract of leaf and fruit of *Terminalia cattapa* in relation to fruit ripening

Sample	Percentage of inhibition (µg/ml)	IC ₅₀ (µg/ml).
Unripe fruit	90.4929 ^a	211.06
Ripe fruit	89.4586 ^b	95.99
BHT (control)	89.2846 ^b	107.61
Leaf	88.9777 ^c	43.34

a, b & cIndicates a significant Different at (P < 0.05)

3.1.3 FRAP Assay

FRAP assay usually measures the ability of antioxidants to reduce ferric complex into blue colour ferrous ion. The result was calculated via the standard curve obtained from Fe (II) sulphate (Table 1). Thus the antioxidant activity of the samples was expressed as equivalence of ferrous sulphate (mM). The FRAP values of the sample used

showed high significant differences between one another at ($P < 0.05$), with leaf extract having higher FRAP value compare to the remaining extract 2512.89 ± 13.47 (mM Fe (II)/g), followed by ripe fruit 1505.11 ± 15.12 (mM Fe (II)/g) and lastly unripe fruit extract 1024.00 ± 14.43 (mM Fe (II)/g) (Table 4)

Table 4: Ferric-reducing antioxidant power of leaf and fruit part *Terminalia cattapa* with respect to fruit ripening

Sample	FRAP value (mM Fe (II)/g)
unripe fruit	1024.00 ± 14.43^c
Ripe fruit	1505.11 ± 15.12^b
Leaf	2512.89 ± 13.47^a

a, b & c Indicates a significant Different at ($P < 0.05$)

3.2 Discussion

3.2.1 Total phenolic and total flavonoids content

The result from this experiment revealed high phenolic and flavonoid content from leaf extract compare to the fruit extract. The phenolic and flavonoid content of leaf extract from the current experiment was found to be higher than the reported value of [21] from methanol and ethyl acetate fraction. Total phenolic content of leaf extract from current research was found to be higher than the reported values of [22] using different sonication treatments, where are total flavonoid content of the current study was higher than 20 minutes sonication and control (with 24-hour maceration) treatment, but lower than 40 minutes sonication and 60 minutes sonication treatment of the same sample. However, the result also revealed that phenolic and flavonoid content, progress with maturity, thus ripe fruit extract showed high amount of both phenolic and flavonoid content than unripe fruit, this contrast the finding of [14], [23] whom reported decrease of phenolic and flavonoid content by maturity of guava fruit and date palm fruit respectively. However, [24 - 25] reported declines in phenolic content by maturity of red raspberries fruit and pomegranate fruit respectively. This differences may be related to the environmental factors, differences of chemical content, and fruits species. Thus *Terminalia catappa* test Sweet to bitter (slightly acidic) where are guava raspberries fruit and pomegranate fruit test sweet to sour (highly acidic) especially at unripe stage.

3.2.2 DPPH antioxidant assay

According to [26] percentage inhibition and IC_{50} are the parameters used to characterise the capability of radical scavenging activity. The lower IC_{50} indicate the higher radical scavenging activity. The result of DPPH antioxidant activity of the samples revealed that fruit extract from both ripe and unripe have higher percentage (%) inhibition than leaf extract, which contrast the report of [9] whom discovered higher antioxidant in leaf compare to fruit extract. Nevertheless unripe fruit showed high percentage of inhibition than ripe fruit, this agree to the finding of [23], [14], [25] whom reported decrease in antioxidant activity by maturity of date palm fruit, guava fruit and pomegranate fruit arils respectively. However, in terms of IC_{50} which is the most important parameter to test antioxidant, leaf showed IC_{50} 43.34 ($\mu\text{g/ml}$) lower than any

of the sample used, including control (BHT), followed by ripe fruit with 95.99 ($\mu\text{g/ml}$), BHT (control) revealed IC_{50} 107.61 lower than unripe fruit. The IC_{50} value obtained from leaf extract of the current experiment was found to be higher than the reported value of [21]. Ripe fruit revealed lower IC_{50} in comparison with unripe fruit. This may be due to increase in vitamin content as the fruit ripening progressed, thus vitamin c also serve as a good antioxidant compounds. However, [27] stated that appropriate management of fruit ripening and control of harvesting time are highly important towards the optimization of antioxidant components as well as nutritional quality of tomatoes.

3.2.3 Ferric-reducing antioxidant Power.

Ferric-reducing antioxidant power assay is established on the basis of reducing of ferric tripyridyl triazine (Fe^{3+} - TPTZ) complex to its ferrous tripyridyl triazine (Fe^{2+} - TPTZ) form and it is a direct assay which measures the quantity of antioxidants from the sample or reducing ability of the sample [22]. The result revealed that leaf extract have higher FRAP value than fruit extract. However, ripe fruit showed higher FRAP value than unripe fruit extract. This corresponds to the finding of [23], [14] whom reported decrease of reducing power by maturity of date palm fruit and guava fruit respectively. This may be associated with the high phenolic and flavonoid content in the leaf extract.

4. Conclusion

Fruit has been use as an antioxidant agent. From the result obtained it can be concluded that all the extract are good source of phenolic and flavonoids content, Nevertheless, leaf show better antioxidant activity, thus it can be used to cure many oxidative illnesses.

5. Future Research

Since many researchers focus on finding natural antioxidant that can be used to cure many oxidative related problems, thus there is also need to study toxicity effect of the samples, for safely consumption, and to identify the functional group as well as chemical structures of the compounds responsible for it antioxidant activity.

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Author's Profile



Abdulaziz Rabiul Abdulkadir is a Faculty of Bioresources and Food Industry, Universiti Sultan Zainul Abidin, (UNISZA), Tembilaka campus Besut, Terengganu, Malaysia