

Total Phenolic Content, Free Radical Inhibition, Antioxidant and Antibacterial Potentials of the Medicinal Organic Compounds in the Fruit of *Terminalia catappa* Linn

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Abstract: Quantitative and qualitative investigation of medicinally active organic compounds in the fruit of *Terminalia catappa* from Nigeria showed that the fruit extract contained several medicinally useful phytochemicals; seventeen components were detected comprising of 98.0 % of the total extract with *cis*-9-hexadecenal (20.0%), 9Z-9-tetradecenal (10.0%), palmitic acid (10.0%), *cis*-9-octadecenal (10.0%), 6Z-6-octadecenoic acid (10.0%), pentadecanoic acid (5.0%), arachidic acid (5.0%) and 1-(+)-ascorbic acid 2,6-dihexadecanoate (5.0%) as the principal components. The fruit extract contains mainly aldehydes (40.0%), fatty acids (33.6%), alkanol (7.2%), ketones (5.6%), ester (5.0%), hydrocarbons (4.6%) and a heterocyclic compound (2.0%) as the major classes of organic compounds. Similarly, the total phenolic content (TPC) was determined to be 3,034 μmg^{-1} gallic acid equivalent. High free radical scavenging and antioxidant potentials were observed in the fruit extract, the results from the free radical scavenging and antioxidant potentials at different concentrations gave inhibitions between 75-88% with IC_{50} : 2.5 μgml^{-1} , this shows that the fruit extract was thrice more active than the synthetic antioxidant (ascorbic acid). The extract showed pronounced sensitivity against two (*S. aureus* and *K. pneumoniae*) out of three bacteria strains tested in this study. The zones of inhibitions were between 19-20mm. The high amount of phenolic compounds leads to more potent radical scavenging, antioxidant and antibiotic properties of the fruit extract. The results concluded that the extracts have a potential source of therapeutic properties of natural origin.

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1. Introduction

Phytochemicals have been utilized as important sources of medicinal drugs and health products. They are important sources of potentially useful compounds for the development of new chemotherapeutic agents because they have a multiplicity of potentially useful actions. Phytochemistry and pharmacomodulation are some the ways used to search for new drugs; research on the secondary metabolites of plants are desirable for the discovery of medicinal potential and to find the actual value of therapeutic uses (Dias *et al.*, 2012; Moghadamtousi *et al.*, 2013). Synthetic drugs are often the option for chemotherapy. However, most synthetic drugs kill not only targeted cells, but also normal cells, and most have severe side effects. There is, therefore, an urgent need for novel treatment options with improved features.

Terminalia catappa Linn (*Combretaceae*) also known as tropical almond is a medium size deciduous medicinal plant. All parts of the plant contains secondary metabolites that are used in traditional medicine such as in management of sickle cell disorders, cancer, rheumatism, diarrhea, dysentery, gonorrhoea and stomach cramps, sexual dysfunction, diaphoretic, antidiabetic, anti-indigestion, anti-

dysentery, anticlstonogenic, stomatitis, skin diseases, arthritis, headache, colic and itching (Muhammad and Mudi, 2011; Akharaiyi *et al.*, 2011). Extracts of this plant have shown activity against drug resistant strains of *Plasmodium falciparum* (Chitmanat *et al.*, 2005). The leaves of the plant are used in aquarium to lower the pH and heavy metal content of water. It has been used in this way by fish breeders for many years, and is active against some parasites and bacterial pathogens. It is also helps to prevent fungus forming on the eggs of the fish (Hnawia *et al.*, 2011).

To best our knowledge, no literature on the total phenolic contents, free radical inhibition, antioxidant and antibacterial potentials of the medicinal organic compounds in the fruit of *T. catappa* Linn have been reported so far. Therefore, it is of great interest to carry out a proper scientific investigation of the fruit extract of this plant in order to validate its medicine properties.

2. Material and Methods

Sample Material

The fruit of the plant was collected from its natural habitat at Ota, Nigeria and it was authenticated as *Terminalia catappa* Linn.

Extraction of the Phytochemicals

The crushed fruit of *T. catappa* (200g) was subjected to solvent extraction using methanol for 3 days. The concentrated crude extract collected was stored in vial at low temperature.

GCxGC-MS Analysis

Analysis of the fruit extract of *T. catappa* was performed using auto-system multi-dimensional GCxGC-MS (Shimadzu, Japan) a data-handling system and a vapourizing injector port, equipped with non-polar and polar double capillary columns (25.0 m x 0.25 μm i.d., 0.25 μm df). High purity helium was used as the carrier gas at a constant flow rate of 0.99 ml/min. A total of 1 μl sample was injected (split ratio 100:1) into GC and GC-MS using AOC20i auto injector for analysis. The initial temperature was set at 60 $^{\circ}\text{C}$, heated at a rate of 3 $^{\circ}\text{C}$ /minutes to 280 $^{\circ}\text{C}$ and held isothermally for 6 minutes. Ion source temperature for these analyses was set at 200 $^{\circ}\text{C}$, while the interface temperature was set at 250 $^{\circ}\text{C}$, solvent cut time was 3.0 minutes and the mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70 eV as acquisition mass range from 40-700 a.m.u. at 0.50 scan/s. The retention indices were determined in relation to a homologous series of *n*-alkanes under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST or with mass spectra from literature.

Determination of Total Phenolic Content

Total phenolic content of the leaf extract of *T. catappa* was determined using the Folin-Ciocalteu method. 1 ml of the methanolic fruit extract was mixed with 46 ml distilled water and 1 ml of Folin Ciocalteu reagent, then 3 ml of (2% w/v) Na_2CO_3 solution was added after 3 minutes and the mixture was allowed to stand for 2 hours for incubation in dark with intermittent shaking, the absorbance of the reaction mixture was measured on a UV-Visible spectrophotometer at 760 nm against a blank (containing all reagents except the test sample). The total phenolic content was expressed as gallic acid equivalents (Settharaksa *et al.*, 2012).

Determination of Free Radical Inhibition and Antioxidant Properties

The 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out for the evaluation of the antioxidant activity. Different concentrations (1000, 100 and 10 μgml^{-1}) of the fruit extract or standards were added to 0.004% w/v methanol solution of DPPH. The mixture was kept in the dark at room temperature for 30 minutes and the

absorbance was measured at 517 nm against a blank (containing all reagents except the test compound). The following equation was used to determine the percentage inhibition (I%) of the radical scavenging activity of the extract.

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

The IC_{50} value ($\mu\text{g/ml}$) is the effective concentration at which DPPH radicals were scavenged by 50% and the value was determined. Where A_{blank} is the absorbance value of the control and A_{sample} is the absorbance value of the test compounds (Khan *et al.*, 2012).

Determination of Antimicrobial Properties

The antimicrobial activities of the fruit extract were determined by agar well method against multi-drug resistance bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. 10 μl of each concentration (1000, 100 and 10 μgml^{-1}) of the fruit extract loaded into well bored and test organism (0.5 McFarland turbidity standard) swabbed agar plates. The plates were incubated at 37 $^{\circ}\text{C}$ for 24 hours. The diameter of the zones of complete inhibition was measured to the nearest whole millimetre (Dhanavade *et al.*, 2011).

3. Results and Discussion

Chemical Composition of *T. catappa* Fruit Crude Extract

GCxGC-MS analysis of the extract of *T. catappa* fruit led to the identification and quantification of a total of 17 major components, accounting for 98.0% of the fruit extract (Table 1). The principal compounds in the extract of *T. catappa* were *cis*-9-hexadecenal (20.0%), 9*Z*-9-tetradecenal (10.0%), palmitic acid (10.0%), *cis*-9-octadecenal (10.0%), 6*Z*-6-octadecenoic acid (10.0%), pentadecanoic acid (5.0%), arachidic acid (5.0%) and 1-(+)-ascorbic acid 2,6-dihexadecanoate (5.0%), while the other nine compounds were present in low concentrations. The fruit extract contains mainly aldehydes (40.0%), fatty acids (33.6%), alkanols (7.2%), ketones (5.6%), ester (5.0%) hydrocarbons (4.6%) and heterocyclic compound (2.0%) as the major class of organic compounds. The chemical compositions of the fruit crude extract of *T. catappa* are different from those reported for the fruit and leaf essential oils; the fruit essential oil contain mainly α -farnesene (21.3%), octadecane (11.7%), palmitic acid (9.5%), 1,2,3-trimethoxy-5-(2-propenyl)benzene (6.6%), neoisothujol (5.8%) and 1,2,4-trimethoxy-5-(1-propenyl)-benzene (4.5%) (Moronkola and Ekundayo, 2000) while leaf essential oil was dominated with (*Z*)-phytol (41.2%), palmitic acid (11.0%), (*E*)-nerolidol (4.7%), heptadecane (3.0%), hexadecane (2.3%), pristane (2.2%) and phytane (2.0%) (Owolabi *et al.*, 2013).

Table 1.0: Chemical Composition, Retention Index and Percentage Composition of the Fruit Extract of *T. catappa*

Compounds	RI	% Composition
4-Methyl-1-penten-3-ol	706	3.6
4-Methyl-1,3-dioxolan-2-one	875	3.6
4,5-Dimethyl-4-hexen-3-one	915	1.0
1,1-Diethoxy-2-butyne	922	1.0
4E-4-Methyl-4-hepten-3-one	938	1.0
Butoxyacetic acid	1050	3.6
1-Propoxyheptane	1091	3.6
5-Hydroxymethylfurfural	1163	2.0
Nitroisobutylglycerol	1444	3.6
9Z-9-Tetradecenal	1609	10.0
cis-9-Hexadecenal	1808	20.0
Pentadecanoic acid	1869	5.0
Palmitic acid	1968	10.0
cis-9-Octadecenal	2007	10.0
6Z-6-Octadecenoic acid	2175	10.0
Arachidic acid	2366	5.0
1-(+)-Ascorbic acid 2,6-dihexadecanoate	4765	5.0
Percentage Total		98.0

Total Phenolic Content (TPC)

The TPC in fruit extract of *T. catappa* was determined by Folin-Ciocalteu's method using UV spectrophotometer. The fruit was characterized high content of phenolics based on fresh weight; this was found to be 3,034 μmg^{-1} gallic acid equivalent. The fruit extract gave a higher TPC when compared with the previous studies on the related species such as methanolic leaf extract of *T. chebula* with TPC value 97.62 μmg^{-1} (Kathirvel and Sujatha, 2012), this shows that the TPC of *T. catappa* is 31 times more than that of *T. chebula*. From the results, it is evident that fruit of this plant is rich in phenolic compounds. The high content of total phenolic compounds indicated that these compounds contribute to the antiradical and antioxidative activities. It has been reported that phenolic compounds show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of phenolic compounds are through scavenging or chelating process. Phenolic compounds are a class of antioxidant agents which act as free radical terminators. Phenolic compounds have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases. The medicinal effects of plants are often attributed to the phytochemical, phenolic content and antioxidant. Plants having significant medicinal values have often been found to be rich in phenolics and to have high antioxidant potentials. The effects of dietary phenolics are of great current interest, due to their antioxidative and possible anti-carcinogenic properties. These compounds that scavenge free radicals help protect against degenerative diseases (Osman *et al.*, 2009).

Free Radical Scavenging and antioxidant Properties

The result of free radical scavenging and antioxidant properties of the *T. catappa* fruit extract was as discussed below. The percentage inhibitions of the fruit extract at various concentrations (1000, 100 and 10 μgml^{-1}) were 88 ± 0.008 , 87 ± 0.004 and $75 \pm 0.002\%$ respectively; while the IC_{50} value was found to be 2.5 μgml^{-1} which was found to be higher than synthetic drug (ascorbic acid) which gave 54 ± 0.002 , 69 ± 0.002 and 96 ± 0.000 as the percentage inhibitions with IC_{50} value of 8.5 μgml^{-1} . The free radical scavenging and antioxidant properties of the extract were found to be greater than synthetic antioxidant, this shows that the fruit extract was about thrice more active than the synthetic antioxidant (ascorbic acid). With the increase of the concentration of the extract, the scavenging activity of the extract increased accordingly. The fruit extract of *T. catappa* possesses efficient scavenging character when compared with the standards and the study reveals that the fruit extract of *T. catappa* exhibited single electron and proton-donating potentials and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The antioxidant property of the fruit extract gave better result at lower concentration when compared with the previous studies on the antioxidant activity on related species such as methanolic extract of *T. belerica* (fruit) 25.11 μgml^{-1} (Alam *et al.*, 2011), *T. chebula* (leaf) 143 μgml^{-1} (Kathirvel and Sujatha, 2012), *T. sericea* (stem-bark) 93.96 μgml^{-1} (Nkolobe *et al.*, 2011). The fruit extract of this plant exhibited high radical termination antioxidant activities. As shown in the equation below the antioxidant present in the extract reacts with DPPH free radical solution and converts them into reduced form either by donating hydrogen atom or transferring single electron followed by proton, where "AH" and "A" are the antioxidant and reduced form of the molecule (Ramani *et al.*, 2012) and this was due to the nature of the organic compounds in the fruit of plant.

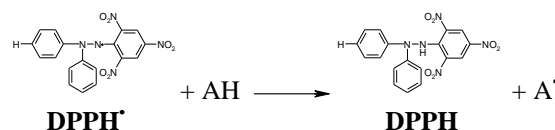


Table 2: Percentage Inhibitions and IC₅₀ of the DPPH Free Radical Inhibition and Antioxidant Properties of the Fruit of *T. catappa* and Reference drug

Extract/ standard	Concentration μgml^{-1}			DPPH IC ₅₀ μgml^{-1}
	1000	100	10	
<i>T. catappa</i>	88±0.008	87±0.004	75±0.002	2.5
Ascorbic acid	96±0.000	69±0.002	54±0.002	8.5

Data are presented as triplicate of the mean \pm S.E.M

Antimicrobial Potentials

The antimicrobial properties of the fruit extract against the representative microorganism examined showed that the fruit extract of this medicinal plant demonstrated better and safe antimicrobial activities compared to synthetic antibiotics, the extract produced excellent inhibition against the two out of three tested bacteria (*S. aureus*, *P. aeruginosa* and *K. pneumoniae*) within the range of 19-20mm as shown in Table 3 below. The highest inhibition zone of 20mm was observed in *S. aureus* and *K. pneumoniae* while there was no inhibition with *P. aeruginosa*. The antibacterial properties of the fruit extract gave a better results at lower concentration when compared with the previous studies on the antimicrobial activities at higher concentration for the leaf water extract of the plant at different stages (young, matured and old red pigmented) on some selected pathogenic bacteria species (*B. cereus*, *S. dysenteriae* and *E. coli*). The old (red pigmented) leaf extract had inhibitory affinity on the tested organisms with halo ranging between 5mm-10mm in diameter, different from the matured leaf extract that ranged in inhibitory halo of between 10.6mm-18.5 mm. The young leaf extract of the plant indeed showed the highest inhibitory effect on the test organisms with halos between 10.2- 20.6 mm. *B. cereus*, *S. dysenteriae* were most inhibited with this extract (20.6 mm) while *E. coli* was the least inhibited (10.2 mm) (Akharaiyi *et al.*, 2011). High antibacterial activities of the fruit extract of *T. catappa* towards methicillin resistant bacteria may be due to the syngenic effect of phytochemicals in the fruit, which exhibited high antibacterial activities. It is worthy to note that the antimicrobial activities of this plant extract were dependent on the rate of diffusion of the extract. These low-molecular weight and highly lipophilic compounds easily diffuse across cell membranes to induce biological reactions. Also, if the extract has high molecular weight, the rate of diffusion is always slow, reduced and also takes longer time, whereas an extract of low molecular weight diffuses faster and at a quicker rate (Orji *et al.*, 2012). The bacteria isolates were found to be sensitive to gentamicin (GEN) only, but resistant to augmentin (AUG) and ceftazidime (CAZ) synthetic antibiotics. The emergence of multi-resistant bacteria strains and the recent appearance of strains with reduced

susceptibility to antibiotics are major sources of concern of health delivery and accessibility due to untreatable bacterial infections and has been correlated with the frequent use of synthetic antibiotics that make the organisms to become resistant to such drug (Baharoglu *et al.*, 2013), because of this reason safe and natural antibiotics were discovered to control the infectious disease causing pathogens. Due to the misuse of antibiotics and an increasing incidence of immunodeficiency-related diseases, the development of microbial drug resistance has become more and more of a pressing problem. Plants having significant medicinal values have often been found to be rich in phenolics and to have high antibacterial potentials. Secondary metabolites in the fruit extract inhibit the life processes of microorganisms by binding their protein molecules, acting as chelating agents, altering their biochemical systems, or causing inflammation of the cells. In this regard the organic compounds in this medicinal plant would play an important role as safe natural antibiotics.

Table 3: Zones of Inhibition (mm) showing the Antimicrobial Potentials of Fruit Extract of *T. catappa* and Reference drugs

Conc. Organism	Fruit Extract				GEN	AUG	CAZ
	Abs	1000	100	10	10 μg	30 μg	5 μg
<i>S. aureus</i>	20	20	20	20	20	-	-
<i>P. aeruginosa</i>	-	-	-	-	11	-	-
<i>K. pneumoniae</i>	20	19	19	19	19	-	-

Key note: - = no inhibition, 6-9 mm = low inhibition, 10-15 mm = moderate inhibition and ≥ 15 mm = high inhibition.

Abs = Absolute

4. Conclusion

These results reveal for the first time that medicinal organic compounds in the fruit of *T. catappa* exert pharmacological properties such as free radical inhibition, antioxidant and antibiotic effects. The study also showed the economic and medicinal benefits arising from the fruit of the plant. The fruit contains significantly high amount of phenolic and therapeutically active compounds which impart excellent medicinal properties. These phytochemicals could increase the shelf life of food products, as well as, they can be used against damaging effects of free radicals and can inhibit degenerative/oxidative disorders, neurodegenerative, carcinogenesis, delay aging and microbial related diseases. The information from this study may direct the pharmaceutical industries to look into the medicinal uses of *T. catappa* fruit.

Conflict of interest statement

We declare that we have no conflict of interest.

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