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PHYTOCHEMICAL SCREENING AND SCAVENGING EFFECTIVENESS OF THE LEAF OF *TERMINALIACATAPPA*LINN

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Abstract: Fresh leaf of *Terminaliacatappa* was collected from Bolori ward Maiduguri Borno state and it was identified by Professor S. S. Sunusi Department of Biological Science Faculty of Science, University of Maiduguri. One thousand grammes (1000g) of the powdered leaf of *Terminaliacatappa* was extracted with methanol using cold infusion (maceration) method. Eighty three point eight two grammes (83.82g) of the dark green in colour gummy in texture of methanol crude extract was obtained, which was further partitioned with n-hexane, ethyl acetate, n-butanol and water to give n-hexane portion (1.638% $^{w}/_{w}$), dark green in colour, oily in texture, ethyl acetate portion (0.075% $^{w}/_{w}$), black in colour, gummy in texture, n-butanol portion (0.777% $^{w}/_{w}$), brown in colour, oily in texture and finally aqueous portion (2.997% $^{w}/_{w}$), dark brown in colour, powdered in texture. Preliminary phytochemical screening of the methanol crude extract and partitioned portions revealed the presence of some secondary metabolites such as cardiac glycoside, flavonoids, saponins, terpenoids, tannins and alkaloid. The antioxidant activity was carried out on the methanol extract and partitioned portions. The methanol extract showed the percentage inhibitions of 98.25 at 10ug/ml 97.40 at 20µg/ml 96.94 at 30µg/ml 96.63 at 40µg/ml and 97.10 at 50µg/ml, n-butanol portion showed the percentage inhibitions of 95.75 at 10ug/ml 96.40 at 20µg/ml 96.15 at 30µg/ml 96.40 at 40µg/ml and 96.15 at 50µg/ml, n-hexane portion showed the percentage inhibitions of 95.50 at 10ug/ml 95.65 at 20µg/ml 95.80 at 30µg/ml 95.75 at 40µg/ml and 95.75 at 50µg/ml, ethyl acetate portion showed the percentage inhibitions of 78.35 at 10ug/ml 87.65 at 20µg/ml 95.00 at 30µg/ml 94.75 at 40µg/ml and 94.70 at 50µg/ml. Finally aqueous portion showed the percentage inhibitions of 94.40 at 10ug/ml 95.10 at 20µg/ml 96.00 at 30µg/ml 95.50 at 40µg/ml and 96.05 at 50µg/ml. The methanol extract showed promising antioxidant activities at various concentrations when compared with the partitioned portions.

INTRODUCTION

*Terminaliacatappa*Linn (Indian almond) is a family of Combretaceae, Usually a small to medium-sized tree 30–50 ft (9–15 m) high and 1 ft (0.3 m) in trunk diameter, but sometimes much larger in diameter and with slight buttresses, evergreen except in areas with a marked dry season. Bark gray, smoothish, thin, becoming slightly fissured. Inner bark pinkish brown, slightly bitter and astringent. Twigs brown, finely hairy when young, slender but swollen at leaf scars and nodes [1]. This plant is commonly called umbrella tree in northern Nigeria because of the shade [2]. In Southeast Asia, leaves and barks of *Terminaliacatappa* are widely used in human as a folk medicine to treat dermatosis, hepatitis, thrush and other oral infections, and intestinal ailments in children. Decoction of the leaves is used to treat indigestion, furred tongue, bronchitis, and tuberculosis [3]. The crushed leaves mixed with coconut oil or coconut cream were used to relieve muscle pain from fractures and sprain [4]. On the other hand, in modern medicine, many pharmacological studies on various extracts of the leaves and barks have been reported to possess anti-cancer [5], antioxidation[6], anti-HIV reverse transcriptase [7], hepatoprotection[8], anti-inflammation [9], aphrodisiac activities [10], antifungal properties againt *Pythiumultimum, Rhizoctoniasolani, Sclerotiumrolfsii*, and *Aspergillusfumigatus*[11], and antibacterial properties against; *Staphylococcus epidermidis*, *S.aureus*, *Bacillus cereus*, *B. subtilis*, and *Pseudomonas aeruginosa* [12]. This plant is also used to treat sickle cell disease in Nigeria and it has been shown to inhibit osmotically induced haemolysis[13]. Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage [14]. Although almost all organisms possess antioxidant defence and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to entirely prevent the damage [15]. However, antioxidant supplements, or foods containing antioxidants, may be used to help human body reduce oxidative damage. Many research studies have revealed that medicinal plants such as *Terminaliaarjuna, Menthaarvensis, Terminaliachebula and Withaniasomnifero* contain various components with antioxidant and anti-microbial activity, which are responsible for their beneficial health effects. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [16]. Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases

[17]. Antioxidant agents of natural origin have attracted special interest because they can protect the human body from free radicals [18]. The aim of this research is to phytochemically screen and determine the scavenging activities of the leaf of *Terminaliacatappa*

EXPERIMENTATION

Sample collection and Identification

Fresh leaf of *Terminaliacatappa* was collected from Bolori ward of Maiduguri Borno State, Nigeria, and identified by a Plant Taxonomist, in the Department of Biological Science, Faculty of Science, University of Maiduguri.

Preparation of the Sample

The plant leaf was freed of extraneous materials material was air-dried in the laboratory at room temperature. The leaf of the plant was ground to fine powder using wooden mortar and pestle and the sample was given a voucher number (562C), stored in the research laboratory of the Chemistry Department, University of Maiduguri for further analysis.

Sample Extraction

The ground leaf material (1,000g) was extracted with 85% methanol using maceration method for 72 hours. The crude extract was concentrated under reduced temperature. The crude extract was then stored in a desiccator.

Partitioning of the Extract

The methanol extract of *Terminaliacatappa*was partitioned with n-hexane until exhaustion. The aqueous fraction was partitioned with ethyl acetate and also partitioned exhaustively with n-butanol. The n-butanol portion and the aqueous portion were then evaporated using rotatory oven. The resulting masses were then weighed and kept in a desiccator for further analysis.

Preliminary phytochemical screening

The methanol crude extract together with the partition portions were subjected to phytochemical screening using standard procedures to identify the constituents [19] [20] [21].

Antioxidant Studies

1,1-Diphenyl l,2- Picrylhydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the extracts was determined using DPPH (Li *et al.,* 2001). The 1,1-Diphenyl l,2- Picrylhydrazyl (DPPH) solution (0.3mM) was prepared in 95% methanol. The sample extracts (1mg) of the *Terminaliacatappa* was mixed with 10ml of 95% of methanol to prepare the stock solution (1mg/ml). Freshly prepared DPPH solution was taken in test tubes and extracts was added followed by serial dilutions (100-1000µg) to every test tube such that the final volume was 2ml and discoloration was measured at 517nm after incubation for 30min in the dark (Spool Matemeter, UV Spectrophotometer, thermo-electron corporation, England, UK). Measurements were performed at least in triplicate. Ascorbic acid 1mg was used as a reference standard and dissolved in DDW to make the stock solution with the same conc. (1mg/ml). The control sample which contained the same volume without any extract and 1ml of 95% methanol was used as the blank. Percent scavenging of the DPPH free radical was measured using the following equation.

DPPH Scavenging Effect % = $(A_0-A_1)/ A_0 \times 100$;

Where, A_0 was the absorbance in the presence of the Grade sample (ethanol and aqueous leaf extract of *Terminaliacatappa).* The actual decrease in absorption induced by the test compounds was compared with the positive control (Mentor *et al.*, 2001). Thin layer chromatography based DPPH assay was performed for qualitative analysis of antioxidants on crude extract and pure isolated portions, TLC was used as stationary phase and three different solvents were prepared as mobile phase. After drying of the sheet, DPPH solution was spread to develop the chromatogram yellow bands were observed which confirmed the presence of antioxidant activity.

RESULTS AND DISCUSSION

Table 1: The Weight, Percentage Yield, Colour and Texture of the Methanol Crude extract and Partitioned portions of *Terminaliacatappa*.

S/NO.	Extract	Weight (g)	Percentage yield (%)$^{w}/_{w}$	Colour	Texture
1.	MCE	83.82	8.382	Dark green	Gummy
2.	NHP	16.38	1.638	Dark green	Oily
3.	EAP	0.75	0.075	Black	Gummy
4.	NBP	7.77	0.777	Brown	Oily
5.	AQP	29.97	2.997	Dark brown	Powdered

Key: MCE = Methanol Crude Extract, AQP = Aqueous Portion, NHP = n-Hexane Portion, NBP= n-Butanol Portion and EAP = Ethyl Acetate Portion.

Table 2: Phytochemical Screening of *Terminaliacatappa* Methanol Crude extract, n-Hexane Portion, Ethyl acetate Portion, n-Butanol Portion and Aqueous Portion.

S/NO.	Test	MCE	NHP	EAP	NBP	AQP
1.	Carbohydrate	+	-	-	+	+
2.	Soluble starch	-	-	-	-	-
3.	Phlabotannins	-	-	-	-	-
4.	Glycosides	-	-	-	-	-
5.	Cardiac glycoside	+	-	-	+	+
6.	Flavonoid	+	-	-	+	+
7.	Terpenoid	+	+	+	-	+
8.	Saponins	+	-	-	-	+
9.	Alkaloid	+	-	-	+	-
10.	Tannins	+	-	-	+	+

Key: MCE = Methanol Crude Extract, NHP = n-Hexane Portion, EAP = Ethyl Acetate Portion, NBP= n-Butanol Portion, AQP = Aqueous Portion, (+) = Present and (-) = Absent.

Table 3: Percentage Inhibition of Standard, Methanol extract, n-Butanol Portion, n-Hexane Portion, Ethyl acetate Portion, and Aqueous Portion of *Terminaliacatappa*

S/No	Extract	10µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml
1	VIT C	99.25±0.05000[a]	98.95±0.05000[a]	98.85±0.1500[a]	96.65±1.850[a]	98.25±0.250[a]
2	ME	98.25±0.1500[a]	97.40±0.2000[a]	96.94±0.03500[a]	96.63±0.035[a]	97.10±0.1000[a]
3	NBP	95.75±0.3500[a]	96.40±0.3000[a]	96.15±0.7500[a]	96.40±1.000[a]	96.15±1.050[a]
4	NHP	95.50±0.9000[a]	95.65±1.550[a]	95.80±1.700[a]	95.75±1.950[a]	95.75±2.150[a]
5	EAP	78.35±0.95[b]	87.65±0.7500[a]	95.00±1.200[a]	94.75±1.450[a]	94.70±0.900[a]
6	AQP	94.40±0.0[a]	95.10±0.3000[a]	96.00±0.1000[a]	95.50±0.1000[a]	96.05±0.150[a]

Key: VIT C = Vitamin C (Ascorbic acid), ME = Methanol extract, NBP = n-Butanol Portion, NHP = n-Hexane Portion, EAP = Ethyl Acetate Portion, and AQP = Aqueous Portion.
a = *P≤0.05* significanceacross the column
b = *P≤0.05* insignificanceacross the column

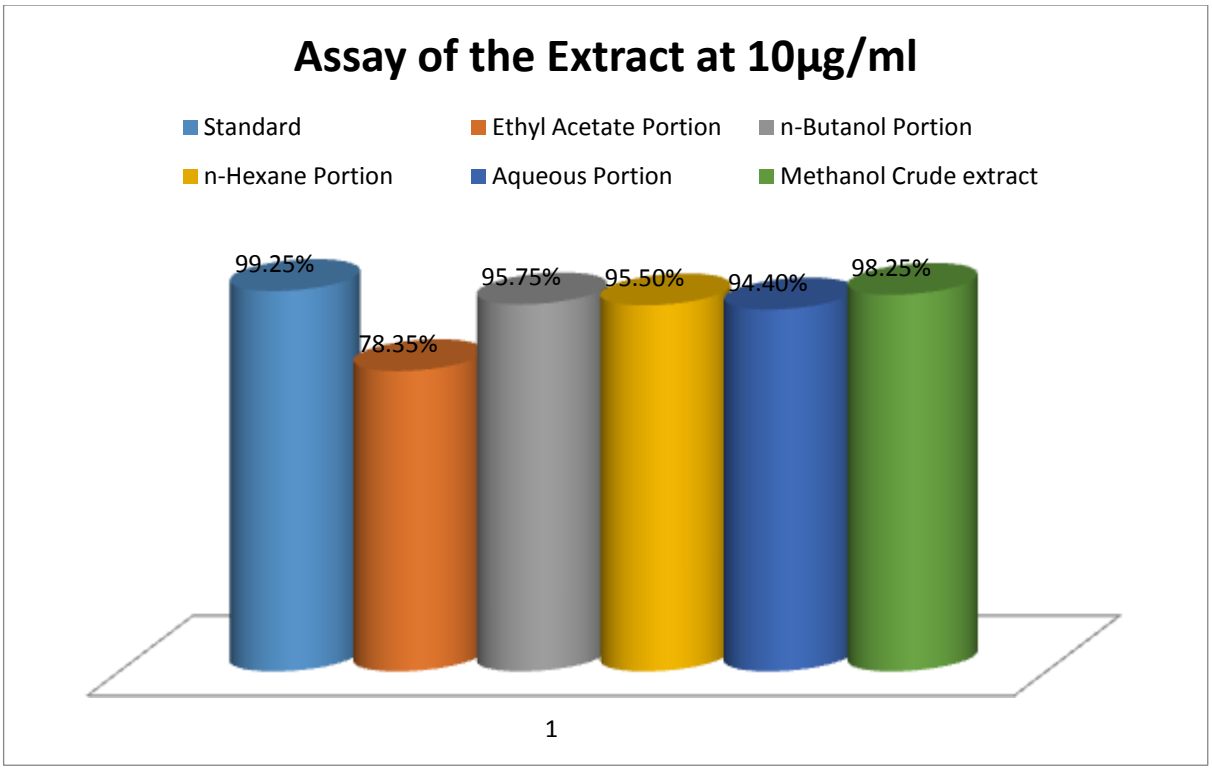

Fig. 1: Antioxidant Property of Methanol extract and Partition Portions of *Terminaliacatappa* at 10µg/ml

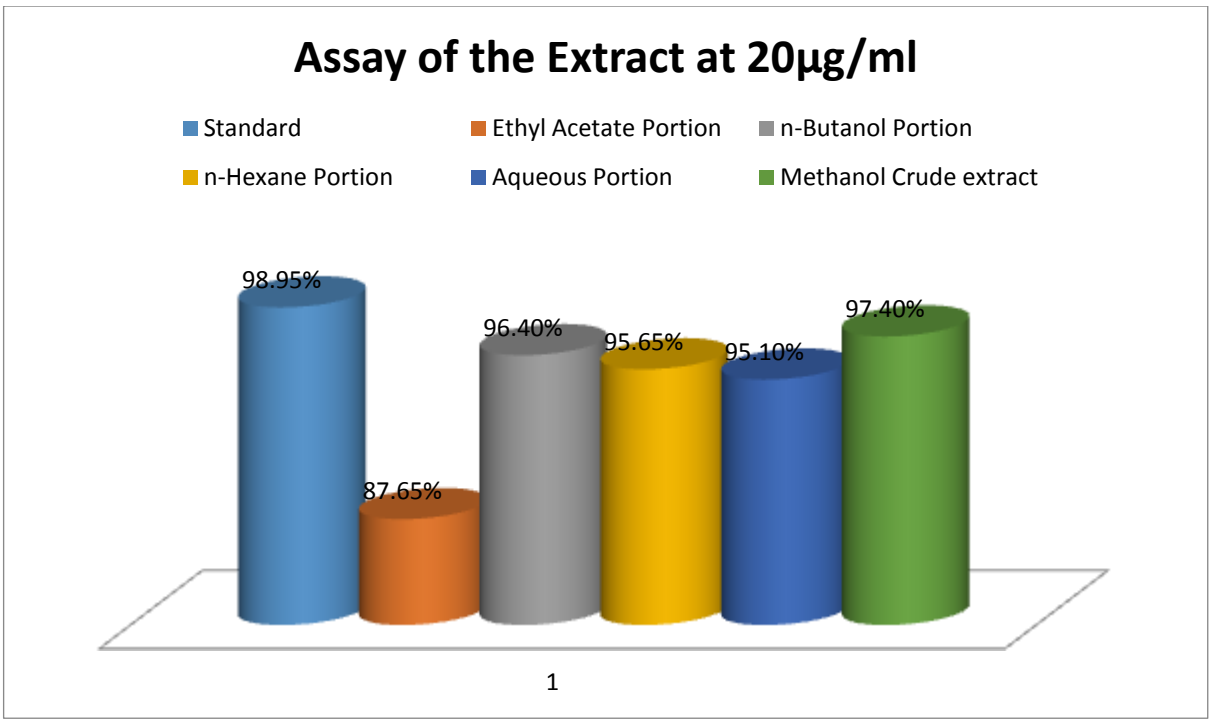

Fig. 2: Antioxidant Property of Methanol extract and Partition Portions of *Terminalia catappa* at 20µg/ml

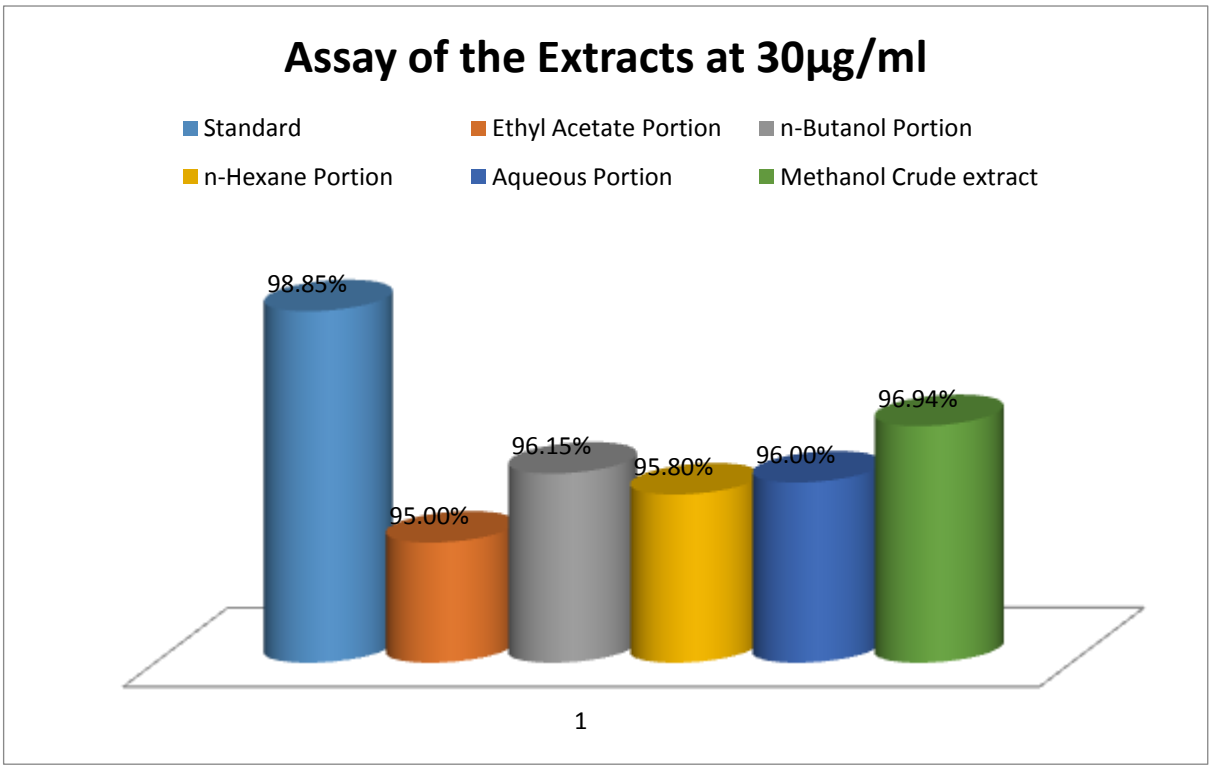

Fig. 3: Antioxidant Property of Methanol extract and Partition Portions of *Terminalia catappa* at 30µg/ml

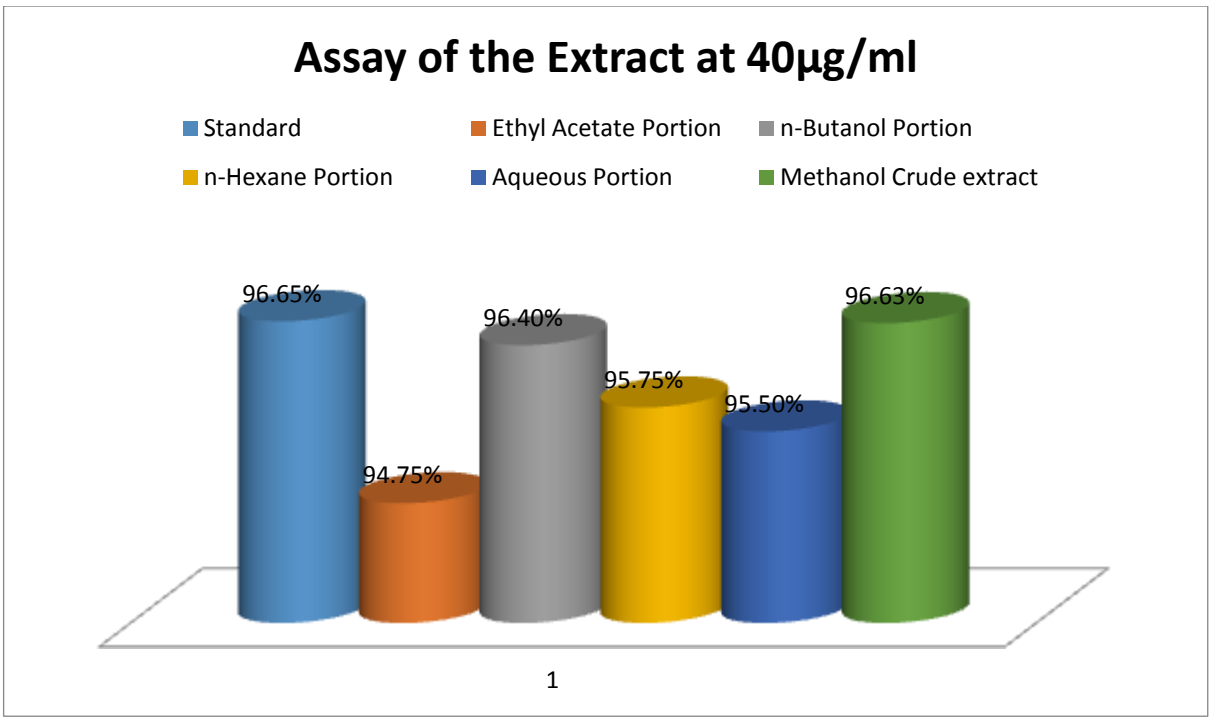

Fig. 4: Antioxidant Property of Methanol extract and Partition Portions of *Terminalia catappa* at 40µg/ml

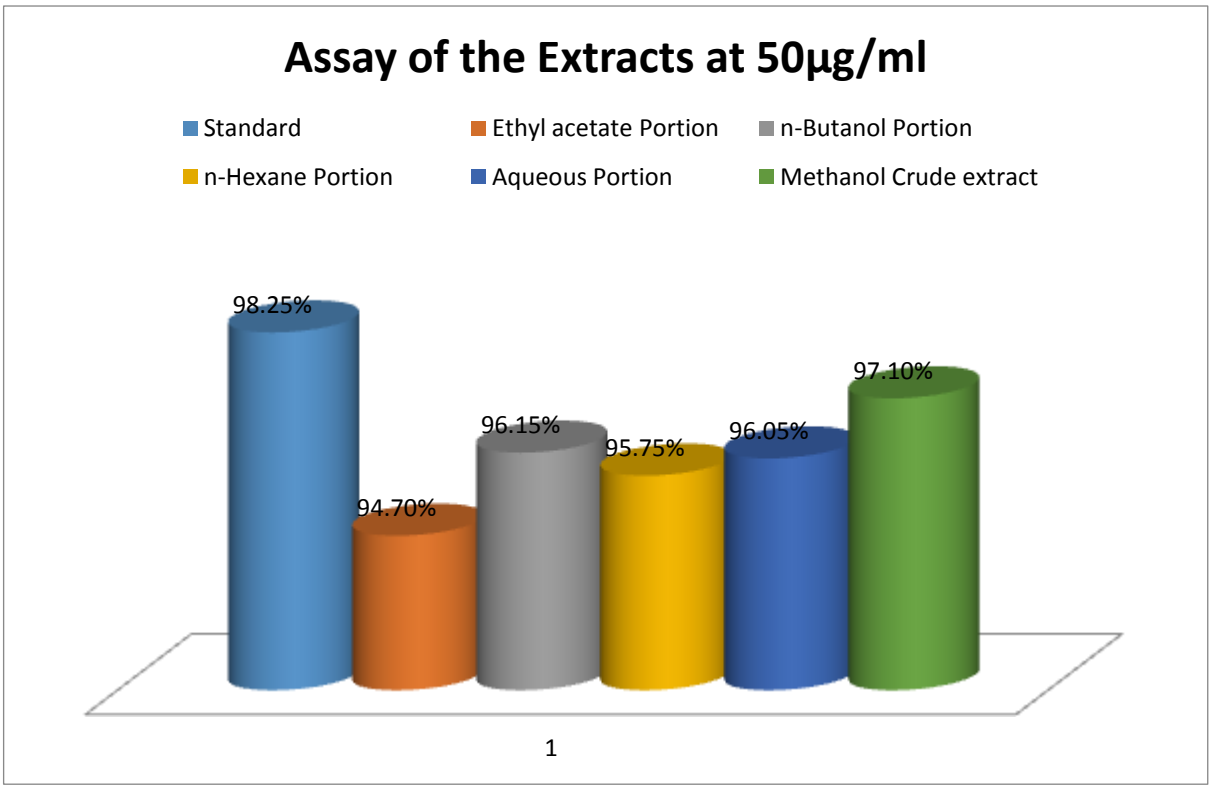

Fig. 5: Antioxidant Property of Methanol extract and Partition Portions of *Terminalia catappa* at 50µg/ml

Plants are rich in phytochemicals that have medicinal and pharmacological values. The methanol crude extract yield 8.382% $^{w}/_{w}$ dark green in colour, gummy in texture, n-hexane portion yield 1.638% $^{w}/_{w}$ dark green in colour, oily in texture, ethyl acetate portion yield 0.075% $^{w}/_{w}$ black in colour, gummy in texture, n-butanol portion yield 0.777% $^{w}/_{w}$ brown in colour, oily in texture and aqueous portion yield 2.997% $^{w}/_{w}$ dark brown in colour, powdered in texture

The results of phytochemical screening of*Terminaliacatappa* leaf extract indicate that the plant contains many secondary metabolites. The methanol crude extract showed the presence of tannins, cardiac glycoside, flavonoid, terpenoidsaponins and alkaloid while glycoside, phlabotannins and were not found in the extract. The *n*-hexane and ethyl acetate partitioned portion showed the presence of terpenoid only, while tannins, cardiac glycoside, flavonoid, saponins, alkaloid, glycoside, phlabotannins and soluble starch were not found. The *n*-butanol partitioned portion was found to contain carbohydrate, tannins, cardiac glycoside flavonoid and alkaloid. However, metabolites such as phlabotannins, terpenoid, saponins, soluble starch and glycosides were not found. Aqueous partitioned portion was also found to contain carbohydrate, tannins, cardiac glycoside, terpenoid, saponins and flavonoid. However, metabolites such as soluble starch, alkaloid, phlabotannins and glycosides were not found. The presence of secondary metabolites in the *Terminaliacatappa* such as tannins, cardiac glycoside, flavonoid, saponnins and Phenolic compound, indicates or implicate the medicinal value of it. These compounds have been reported to have antioxidants property and exhibit a wide range spectrum of medicinal properties such as anti-cancer, anti-inflammatory and anti-diabetes [22] [23]. Those plants that contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, and tannins), nitrogeneous compounds (such as alkaloids and amines), vitamins, terpenoids (including carotenoids) and some other metabolites are found to be reached in antioxidant activity [24].

The results of antioxidant evaluation showed that the methanol crude extract as well as the partitioned portions indicates promising antioxidant properties of leaf of *Terminaliacatappa,* the reducing power of the extracts compared with the reference standard antioxidant drug (ascorbic acid). The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The methanol extract

showed the percentage inhibitions of 98.25% at 10ug/ml 97.40% at 20µg/ml 96.94% at 30µg/ml 96.63% at 40µg/ml and 97.10% at 50µg/ml, n-butanol portion showed the percentage inhibitions of 95.75% at 10ug/ml 96.40% at 20µg/ml 96.15% at 30µg/ml 96.40% at 40µg/ml and 96.15% at 50µg/ml, n-hexane portion showed the percentage inhibitions of 95.50% at 10ug/ml 95.65% at 20µg/ml 95.80% at 30µg/ml 95.75% at 40µg/ml and 95.75% at 50µg/ml, ethyl acetate portion showed the percentage inhibitions of 78.35% at 10ug/ml 87.65% at 20µg/ml 95.00% at 30µg/ml 94.75% at 40µg/ml and 94.70% at 50µg/ml. Finally aqueous portion showed the percentage inhibitions of 94.40% at 10ug/ml 95.10% at 20µg/ml 96.00% at 30µg/ml 95.50% at 40µg/ml and 96.05% at 50µg/ml. However the activity of the extracts is lower when compared to the standard antioxidant drugs (ascorbic acid). It was observed that the scavenging activity of ethyl acetate fraction was insignificant at concentration of 10µg/ml. In this research experiment it could be observed that a total inhibition not achieved with ranges from 78% – 96% which is in similar findings of [25] [26] [27]. Although, a total scavenging of free radicals was not achieved, however significant scavenging was recorded when compared to the standard (vitamin C).

The scavenging activities of methanol extract and partitioned portions of *Terminaliacatappa* methanol leaf extract at different concentrations of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50µg/ml. However, across all concentrations, methanol extract and n-butanol extract showed the highest scavenging activity (antioxidant activity) than the other portions when compared to the standard (Ascorbic acid). This high activity could be attributed due to presence of phenolic compounds [28] such as flavonoids and tannins which are presence in this study. The antioxidant activity of phenolic compounds is mainly due to their ability to undergo redox reaction; this plays an important role in neutralizing reactive oxygen species or free radicals [29].

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