



## Cytotoxic Correlation of Selected Nigerian Ethnomedicinal Plants Using Brine Shrimp Lethality Test

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**ABSTRACT:** Cytotoxicity is the toxicity caused due to the action of chemotherapeutic agents on living cells and its mechanism lies in the ability of chemotherapeutic agents to oxidatively attack vital components inside cells, creating reactive free radicals that can cause adverse effects in the nucleus, proteins, and/or lipids. There are several ethnomedicinal plants in Nigeria used in the amelioration of several illnesses, however, information on their cytotoxic assessments are rare. Hence, the present study was to assess the cytotoxic correlation of selected Nigerian ethnomedicinal plants using brine shrimp lethality test. Data show that calculated LC<sub>50</sub> of the extracts ranged from 83.71-265.90 µg/cm<sup>3</sup>, with *K. africana* having the highest anticancer activity with LC<sub>50</sub> 83.71 µg/cm<sup>3</sup>. The present result is an indicative of the presence of cytotoxic compounds that are attributable to the bioactive components with high phenolic contents derived from plant parts (fruit, leaf, seed) that accounted for the pharmacological effects or potential against various human diseases such as cancer. The cytotoxicity for the various plant parts' extracts showed concentration dependent manner and it was determine candidate plants for isolation of cytotoxic principle.

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Plants as natural resources have served mankind as an important source of food and medicines (Chen *et al.*, 2020). Over decade, man has exploited some of these plants as herbal medicines in the treatment of numerous diseases and people have developed interest in searching empirical information regarding the therapeutic values of local plants before the advent of orthodox medical treatment began (Mehra *et al.*, 2021). Medicinal plants are the oldest source of medicaments with great efficacy in combating many diseases (Tahir *et al.*, 2022). It has been estimated that 80 % of the inhabitants of the world rely mainly on medicinal plants for their primarily health care needs. This indicates that major part of traditional healings involves the use of plant extracts or their active

principles (WHO, 2020). Plants are rich in a wide variety of secondary metabolites capable of combating different kind of ailments including infectious diseases. Over the years, many synthetic drugs have been reported to have displayed some side effects and toxicity, amongst other problems (Yuan *et al.*, 2016). This necessitates the search for new lead compounds for the remedies of various diseases (Ved *et al.*, 2020). In recent times, there has been resurgence of interest in natural products as major source of medicines of novel molecules for use in the elucidation of physiological and biochemical principles (Alves, 2020; Freitas *et al.*, 2021). It has been reported that plant based extracts use as remedies are safer, cheap and have less side effects than synthetic drugs (Morsy,

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2019). Natural products discovered from medicinal plants have provided numerous clinically used medicines that have proven to be more reliable (Tan *et al.*, 2020). Several plants including; *Kigelia africana* (*K. africana*), *Calotropis profera* (*C. profera*), and *Jatropha curcas* (*J. curcas*), have reportedly been used ethnomedicinally in the treatment of cancer and other related diseases in Nigeria and other African Countries. However, Literature revealed that majority of these plants has not been adequately screened for their anticancer properties using conventional approach. Therefore, these three plants (*Kigelia africana*, *Calotropis profera*, and *Jatropha curcas*) candidate were selected based on the authentication and taxonomical information obtained. Hence, the objective of this paper is to evaluate the cytotoxic correlation of selected Nigerian ethnomedicinal plants using brine shrimp lethality test.

## MATERIALS AND METHODS

**Sample Preparation and Extraction:** Fresh fruit, leaf, and seeds of *Kigelia africana*, *Calotropis procera*, and *Jatropha curcas*, respectively were collected from a farmland in Suleja Local Government Area, Niger State, in the month of May, 2023. A plant taxonomist; Mr. Akeem Adeyanj Lateef, duly identified the plants, and deposited them with voucher numbers: NIPRD/H/7326, NIPRD/H/ 7328, and 7327 respectively at the Herbarium and Ethnobotany unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute of Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. The fresh parts of each plant were separately cut into pieces, air-dried at room temperature. The dried samples (*K. africana*, *C. procera*, and *J. Curcas*,) were then pulverized prior use. 500 g of air-dried and pulverized plants parts were separately subjected to three consecutive rounds of cold maceration using 500.0 cm<sup>3</sup> each of 70 % methanol for 48 hrs at room temperature, followed by filtration using Whatman grade No. 1 filter paper. The resulting solution was decanted, filtered and then concentrated under reduced pressure using rotary evaporator. The concentrates were evaporated to dryness at 40 °C using *vacuo* on a water bath, air dried to constant weight (Abubakar *et al.*, 2015a; Tanko *et al.*, 2019). The extracts were coded; MKaF, MCpS, and MJcL for methanol crude *Kigelia africana*, *Calotropis procera*, and *Jatropha curcas* extracts respectively.

**Phytochemical Screening:** The phytochemical screening of the plants extracts was carried out using standard methods:

**Test for alkaloids (Dragendorff's test):** Few drops of Dragendorff's reagents were added to MKaF, MCpS, and MJcL each. Formation of red precipitate confirms the presence of alkaloids (Abubakar *et al.*, 2015b; Jabeen *et al.*, 2023).

**Test for anthraquinones (Borntrager's test):** 3 cm<sup>3</sup> of MKaF, MCpS, and MJcL each was shaken with 3cm<sup>3</sup> benzene; filtered and about 5 cm<sup>3</sup> of 10 % ammonia solution was added to the filtrate. The mixture was shaken once more and the present of a pink, red or violet coloration in the ammoniacal (lower) phase indicates the presence of anthraquinone derivatives (Omotanwa *et al.*, 2023).

**Test for flavonoids (alkaline reagent test):** To MKaF, MCpS, and MJcL each, 0.1 g each was be mixed with a few drops of dilute NaOH. Formation of intense yellow colour which becomes colourless on the addition of dilute HCl indicates the presence of flavonoids (Abdul-Basit *et al.*, 2023).

**Test for phenolic compounds:** To MKaF, MCpS, and MJcL each, 0.1 g each was dissolved in 2 cm<sup>3</sup> of distilled water and 3 drops of 10% ferric chloride was added to the mixture. Formation of blue or green colour indicates presence of phenols (Abdul-Basit *et al.*, 2023).

**Test for phlobatannins:** To MKaF, MCpS, and MJcL each was be boiled with dilute HCl solution to produce a red precipitate, indicating the presence of phlobatannins (Gabrewbet *et al.*, 2023).

**Test for saponins (Froth Test):** To MKaF, MCpS, and MJcL each, about 20 cm<sup>3</sup> of distilled water was agitated in a graduated cylinder for 15 minutes. The formation of about 1 cm<sup>3</sup> layer of foams indicates the present of saponins (Rai *et al.*, 2023).

**Test for steroidal compounds (Liebermann-Burchard's test):** 2 cm<sup>3</sup> of acetic anhydride was added to 0.5 g of MKaF, MCpS, and MJcL each, and then transfer to a test tube containing 2 cm<sup>3</sup> H<sub>2</sub>SO<sub>4</sub>. The colour changed from violet to blue, or green in the samples which indicates the presence of steroids, or formation of brown ring at the junction indicating the presence of steroids (Omotanwa *et al.*, 2023).

**Test for tannins:** About 0.5 g of each dried pulverized *K.africana* fruit, *C. procera* seed, and *J. Caucis* samples each was boiled in 20 cm<sup>3</sup> of distilled water in a test tube and then filtered. A few drops of 0.1 % ferric chloride was be added, a brownish-green or a blue-black colouration was formed, indicating the presence of tannins (Peiris *et al.*, 2023).

**Test for terpenoids (Salkowski's test):** The MKaF, MCpS, and MJcL each, 0.1 g was dissolved in chloroform (2 cm<sup>3</sup>) and filtered. Few drops of concentrated H<sub>2</sub>SO<sub>4</sub> were added to the filtrate. Formation of reddish brown interphase indicates the presence of terpenes (Shaba *et al.*, 2013; Alhaithloul, 2023).

**Quantitative Determination of the Phytochemical Constituents:**

**Preparation of fat-free sample:** 2 g of the samples each was defatted with 100 cm<sup>3</sup> of diethyl ether using a Soxhlet apparatus for 2 hours.

**Determination of total phenols:** The fat free MKaF, MCpS, and MJcL each was boiled with 50 ml of ether for the extraction of the phenolic compounds for 15 min. 5 cm<sup>3</sup> of each extract was pipetted into a 50 cm<sup>3</sup> flask, and then 10 ml of distilled water was added. 2 cm<sup>3</sup> of ammonium hydroxide solution and 5 cm<sup>3</sup> of concentrated alcohol were also added. The samples each was made up to mark and was allowed to react for 30 min for colour development. Each was measured at 505 nm (Tsado et al., 2018).

**Alkaloid determination:** 5 g of MKaF, MCpS, and MJcL each was weighed into a 250 cm<sup>3</sup> beaker and 200 cm<sup>3</sup> of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. Each was then filtered and the filtrate was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Abdul Basit et al., 2023).

**Tannins determination:** 500 mg MKaF, MCpS, and MJcL each was weighed into a 50 cm<sup>3</sup> plastic bottle. 50 cm<sup>3</sup> of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 cm<sup>3</sup> volumetric flask and was made up to the mark. Then 5 cm<sup>3</sup> was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl<sub>3</sub> in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes (Paramesha et al., 2023).

**Saponins determination:** To pulverized *K. africana* fruit, *C. procera* seed, and *J. Curcas* leaf each, 20 g of each was put into a conical flask and 100 cm<sup>3</sup> of 20% aqueous ethanol was added. Each sample was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. Each mixture was filtered and the residue was re-extracted with another 200 cm<sup>3</sup> 20% ethanol. The combined extracts each was reduced to 40 cm<sup>3</sup> over water bath at about 90 °C. The concentrate was transferred into a 250 cm<sup>3</sup> separating funnel and 20 cm<sup>3</sup> of diethyl ether was added and shaken vigorously. The aqueous was recovered while the ether layer was discarded. The purification process was repeated. 60 cm<sup>3</sup> of n-butanol was added. The combined n-butanol extract each was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the each sample was dried in the oven to a

constant weight; the saponin content was calculated as percentage (Sharma et al., 2023).

**Flavonoid determination:** 10 g of pulverized *K. africana* fruit, *C. procera* seed, and *J. Curcas* leaf each was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Tanko et al., 2017).

**Brine Shrimp Lethal Test:** The cytotoxicity of the plants extracts were investigated using Brine shrimp lethal assay. *Artemia salina* (*A. salina*) (1 g) cyst were incubated for hatching in a transparent container filled with 3.3 % artificial sea water with constant exposure to light for 48 hrs. The hatched *A. Salina* was collected using pipetting into vials used for bioassay. Stock solution of each extracts (10, 000 µg/cm<sup>3</sup>) were made in 5 % dimethyl sulphoxide (DMSO) from which concentrations were prepared for each extract. *A. salina* (10 g) were counted macroscopically in the stem of a Pasteur pipette against a lighted background and transferred into each sample vial containing different concentrations of each extract (15.63, 31.25, 62.50, 125.0, 250.0, and 500.0 µg/cm<sup>3</sup> prepared with 3.3 % artificial sea water). A drop of dry yeast suspension was added as food to each vial. All vials were maintained under illumination. The surviving nauplii were counted with the aid of 3x magnifying glass after 24 hrs. The mean mortality for each of the extract was determined. The surviving nauplii were killed by addition of 100 µL of 5 % (v/v) phenol to each tube. Each procedure was carried out in triplicates. LC<sub>50</sub> values of the Brine shrimps afforded from the extracts of the plants were recorded after subjecting the values in 95 % confidence intervals by analyzing the data with statistical analysis.

## RESULTS AND DISCUSSION

The results of the percentage yield of the crude methanol extracts of *K. africana* fruit, *C. procera* seed, and *J. Curcas* leaf are presented in Table 1.

**Table 1:** Percentage yield of the Methanol Crude Extracts of the Plants Samples

Extracts	Weight (g)	Yield (%)
MKaF	50.66	10.13
MCpL	40.80	8.16
MJcS	34.22	6.84

MKaF = Methanol crude extract of *Kigelia africana* fruit, MCpL = Methanol crude extract of *Calotropis procera* leaf, and MJcS = Methanol crude extract of *Jatropha curcas* seed.

From the results, it was revealed that out of 500 g each of the pulverized samples, the yield of the crude methanol extracts differs significantly. The highest yield was obtained from the fruit of *K. africana* (50.66 g; 10.13 %) while *C. procera* leaf and *J. Curcas* seeds yield (40.80 g; 8.16 %) and (34.22 g; 6.84 %)

respectively. This difference may be attributed to morphological variations of the plants.

**Table 2:** Qualitative phytochemical screening of the methanol crude extracts of the plants samples.

Phytochemical	MKaF	MCpS	MJcL
Alkaloids	+	-	+
Anthocyanins	+	-	-
Anthroquinones	+	+	-
Flavonoids	+	+	+
Phenols	+	+	+
Phlobatannins	-	-	-
Saponins	+	-	+
Steroidal compound	-	-	-
Tannins	+	+	+
Terpenoids	-	-	-

+ = present, - = absent; MKaF = Methanol crude extract of *Kigelia africana* fruit, MCpL = Methanol crude extract of *Calotropis procera* leaf, and MJcS = Methanol crude extract of *Jatropha curcas* seed.

Extraction carried out by Arkhipov *et al.* (2014) from *Kigelia africana* leaf reported that out of 1 g of the

dried sample, methanol crude extracts afforded 199 mg. The results of the phytochemical screening of the samples are presented in Table 2 Various phytochemical constituents were detected from the plants' samples.

The crude methanol extracts of the plants (MKaF, MCpS, and MJcL) for *K. africana* fruit, *C. procera* seeds and *J. Curcas* leaf respectively, revealed the presence of some secondary methabolites including; alkaloids, anthocyanins, anthoquinoes, Flavonoids, Phenols, saponins, and Tannins. Khan and Islam (2012) and Arkhipov *et al.* (2014) reported the presence of several phytochemicals in *Kegelia pinnata* leaf and fruit including; Alkaloids, glycosides, gums, reducing sugars, tannins, flavonoids, and saponins. The quantitative determination of some phytoconstituent in the three plants sample revealed a good quantity of the phytoconstituents as presented in Table 3.

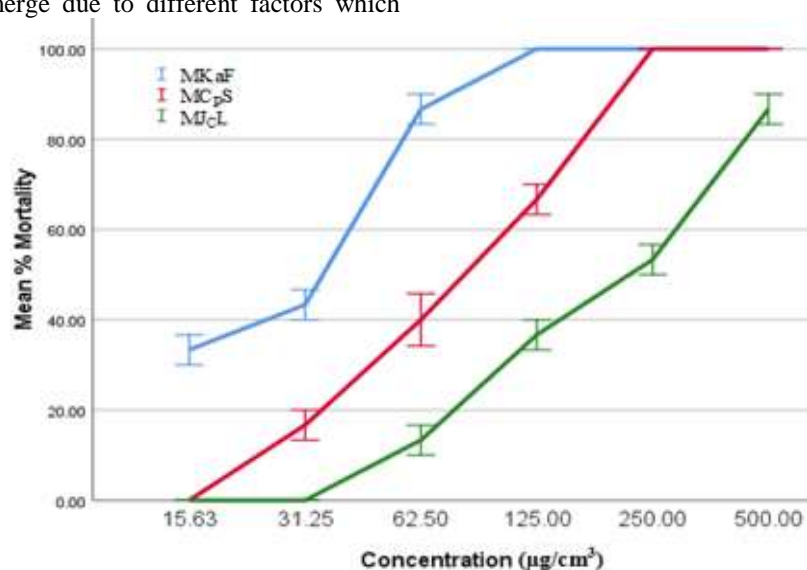
**Table 3:** Quantitative phytochemical screening of the methanol crude extracts of the selected plants' samples (mg/100 g)

Sample	Phenol	Flavonoids	Tannins	Saponins	Alkaloids
MKaF	634.64	121.18	154.23	33.88	50.37
MCpS	287.69	67.10	94.92	52.10	31.25
MJcL	207.46	25.25	44.33	21.53	16.85

MKaF = Methanol crude extract of *Kigelia africana* fruit, MCpL = Methanol crude extract of *Calotropis procera* leaf, and MJcS = Methanol crude extract of *Jatropha curcas* seed.

From the results, it was revealed that methanol extract yielded high amount of phenolic compounds in all the plants samples. Methanol crude extract of *K. africana* leaf (MKaF) yields highest amount of phenolic compounds such as; phenols, flavonoids, tannins with values (mg/100 g) 634.64, 121.18, and 154.23 respectively. Cancer is a disease in which abnormal cells divide uncontrollably and destroy body tissues (Insamran and Sangrajiran, 2020). Cancer develops through accumulation of genetic changes or mutations which could emerge due to different factors which

amongst others include; physical (such as; ultraviolet (UV) radiations), chemical (such as; chewing and smoking of tobacco, chemical pollutants/mutagens), biological (such as viruses) and in some cases, it may be through hereditary (Gupta *et al.*, 2017). The results of cytotoxic lethality test of the methanol crude extracts of the three selected plants displayed cytotoxic activity against Brine shrimp with IC<sub>50</sub> values; 83.71, 144.02, and 265.90 µg/ml for MKaF, MCpS, and MJcL, respectively (Fig. 1).



**Fig. 1:** Brine shrimp test of cytotoxicity of the methanol crude extracts of the plants samples at different concentrations (µg/cm³). MKaF = Methanol crude extract of *Kigelia africana* fruit, MCpL = Methanol crude extract of *Calotropis procera* leaf, and MJcS = Methanol crude extract of *Jatropha caucas* seed.

This difference in the toxicity may be attributed to the chemical complexity of the crude extracts with bioavailability of the active phytoconstituents in the plants samples (Asoso *et al.*, 2019). These values were similar to the LC<sub>50</sub> values; 50 and 300 (µg/ml) for *Kigelia pinnata* (Khan and Islam, 2012); 214 and 334.10 (µg/ml) for *C. procera* methanol stem crude extract (Asoso *et al.*, 2019). However, values were higher than 26.02 and 55.01 µg/ml for toxicity tests of *K. africana* methanol stem bark extracts (Mukavi *et al.*, 2020). This indicates that MKaF had the highest cytotoxic activity than the others due to its activities at lower value of LC<sub>50</sub>. At higher concentrations the plants exhibited cytotoxic activities, an indication that the activities were based on dose dependent manner (Fig 1). This was proven as the mortality increased gradually increased in the percentage of the mortality rate with increase in concentration of the extracts. Study conducted by Asoso *et al.* (2019) reported that mortality increased with increased in the concentration of the extracts. An extract with LC<sub>50</sub> greater than 1000 µg/ml in the Artemia nauplii bioassay have been reported to be non-toxic (Halder and Sharma (2017). This activity exhibited by MKaF could be attributable to its endowed phenolic compounds. MJcL had the least potent among the three samples tested against the cancer cells as compared to others since the Brine shrimp mimics cancer cells behaviour. Phenolic compounds have been reported for their cytotoxic and potent anticancer activity (Blanco-Vaca *et al.*, 2019; Costea *et al.*, 2019; Miyata *et al.*, 2019).

**Conclusion:** Cytotoxic correlation of selected ethnomedicinal plants using Brine Shrimp lethality test revealed the presence of various phytochemicals. The present result is an indicative of the presence of cytotoxic compounds that are attributable to the bioactive components with high phenolic contents derived from plant parts (fruit, leaf, seed) that accounted for the pharmacological effects or potential against various human diseases such as cancer. *Kigelia africana* fruit extract had the highest phenolic compounds compared to *Calotropis procera* seeds and *Jatropha curcas* leaf extracts.

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