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Extraction, Maceration and Assessment of Potential toxicity of  
Crude Ethanol Leaf-Extract and Fractions of  
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## ABSTRACT

*Naucleadiderrichii* is widely used in folk medicine in the treatment of infectious diseases in Mada District, Gusau, Zamfara State, Nigeria and other parts of Africa. This study deals with the extraction, maceration and evaluation of the level of toxicity in crude ethanol leaf-extract and other fractions of the crude using *Artemiasalina* (brine shrimp) model. The crude leaf-extract (Nd-CETE), was extracted using absolute ethanol, while the fractions (Nd-PETF, Nd-CHLF and Nd-EAF) of the crude were obtained through maceration using solvents of varying polarity gradient (petroleum ether, chloroform and ethyl acetate). The lethality of the Brine shrimp nauplii was in a concentration dependent fashion, with increasing lethality due to increase in concentration of the crude extract/fractions. The Lethality of crude extract and those of fractions were based on Meyer's index of toxicity. Fractions of petroleum ether and Chloroform were found to be non-toxicity with  $LC_{50}$  3,239.09 and 1,574.60  $\mu\text{g/mL}$  respectively, while the crude ethanol leaf-extract (Nd-CETE) and ethyl acetate fraction (Nd-EAF) demonstrated high levels of toxicity in *Artemiasalina* with  $LC_{50}$  342.90 and 578.08  $\mu\text{g/mL}$  respectively, which signify toxicity in a brine shrimp model. The study concludes that, the crude leaf-extract and ethyl acetate fraction of *Naucleadiderrichii* possesses cytotoxic behavior, suggesting the presence of potential bioactive chemical components. Further *in vivo* and cell lines cytotoxicity test is recommended to substantiate these findings.

**Keywords:** *Naucleadiderrichii*, Extraction, Maceration, toxicity, *Artemiasalina*, lethality.

## 1.0 INTRODUCTION

The natural environment is furnished with plants and plant's products which have been found to be of great medicinal and nutritional importance (Bonaventure *et al.*, 2017). There are many experimental pieces of evidence indicating the use of plants for medicinal purposes, hence, the plant kingdom has become a target for the search of biologically active compounds for the complementary/alternative management of human ailments (Osigwee *et al.*, 2015). These plant products produced, either as pure compounds or standardized extracts, provide unlimited opportunities for new drug leads, because of the unmatched availability of chemical diversity (Sasidharan *et al.*, 2011).

Active compounds produced during secondary metabolism are usually responsible for the biological properties of plant species used throughout the globe for various purposes, including treatment of infectious diseases (Singh, 2015 and Refazet *et al.*, 2017). They are produced either as a single compound or in combination as a group of related compounds within the natural plants' products (extract/fraction) and as potentials for drug discovery (Soderberg, 2019).

*Naucleadiderrichii* is an evergreen tree belonging to the Rubiaceae family. It is endemic in West and Central Africa and has been extensively studied for its anti-malaria (Lamidiet *et al.*, 1996), anti-plasmodial (Mustofaet *et al.*, 2000), anti-leishmanial (Di

Giorgio, 2006) and anti-trypanosomal (Nwodoet *al.*, 2010) activities.

In folkloric medicine in Nigeria and other part of the world, particularly West Africa, *Naucleadiderrichii* is used in the management of a wide range of ailments ranging from endocrine disorders, infections, wound healing, anti-malaria, anemia, stomachache, indigestion, fever and jaundice. In Nigeria, bark preparations are used against fever and malaria, and as antiperiodic, appetizer and others (Orwaet *al.*, 2009). It is also used for its anti-diabetic activity with good toxicological profile (Bonaventureet *al.*, 2017). The bark and leaf decoctions of the plant are taken in Sierra Leone and Ghana against stomachache, diarrhea, malaria, as a wash for measles and as a foot wash after long walks. In Côte d'Ivoire, the bark is sometimes used to treat bacterial infection. In Gabon, a bark infusion is drunk against fever. In Congo, bark decoctions are taken or the leaf pulp is rubbed in for the treatment of fever, stomach problems, gonorrhea, hepatitis and menstruation problems, while a bark infusion is taken as a vermifuge. In various countries leaf infusions, leaf decoctions and the leaf pulp are drunk or used in washings, baths or embrocation to treat fever. In Guinea leaf preparations are applied on tumours. The ripe infructescence is also eaten as a cough medicine in Sierra Leone (*Naucleadiderrichii* (PROTA), 2015).

The aim of this research is to extract, macerate and evaluate the potential toxicity effects of crude ethanol leaf-extract (Nd- CETE) and three fractions (Nd-PETF, Nd-CHLF and Nd-EAF) of *Naucleadiderrichii* at different concentrations.

## 2.0 MATERIALS AND METHODS

### 2.1 Botanic Description of the Plant (*Naucleadiderrichii*)

*Naucleadiderrichii* is a plant with English name (African peach) and trade name (Opepe). It is an evergreen tree that reaches a height of 30-40 m and a diameter of 0.9-1.5 m; bole cylindrical, slender, straight and branchless and a broad spherical crown with thick foliage. The shining leaves are 15 cm long and bigger when young, elliptic, and acute at the ends, keeled towards the base, and stipulate, with a pair of distinct leafy stipules at the base. It is mostly deciduous except at the ends of shoots

and the nodes are often occupied by ants. The Flowers are small, green-white-yellow and tubular, in solitary terminal heads (unbranched), 3 cm across; stalks only about 1 cm. The fruit is yellow, fleshy, in a globose head deeply pitted between the deeply fused calyx lobes (Orwaet *al.*, 2009).

### 2.2 Traditional Description of the plant (*Naucleadiderrichii*)

The plant, *Naucleadiderrichii* is commonly known locally as Opepe (Nigeria), Opepi (United Kingdom), Kusia (Ghana), Badi (Ivory Coast), Bilinga (Gabon), Akondoc (Cameroon), Kilingi (Uganda). It is a medium sized tree up to 30 m tall with bole up to 80 cm in diameter. The Heartwood is orange or golden yellow, darkening on exposure; sapwood whitish or pale yellow, clearly defined. Texture rather coarse; grain usually interlocked or irregular; lustrous; without characteristic odor or taste (*Naucleadiderrichii* (PROTA), 2015).

### 2.3 Sample Collection and Authentication

Fresh leaf samples were plucked directly from the plant at Mada district, Gusau, Zamfara State, Nigeria. The plant was identified and authenticated as *Naucleadiderrichii* specie with a common name, Opepe plant and a local name (Hausa), Tuwanbiri. The identification and authentication were done at the Department of Plant Biology, Bayero University, Kano, Nigeria, after which the plant was given Herbarium Accession Number, 0434.

### 2.4 Sample Preparation

The fresh leaves of *Naucleadiderrichii* were washed with clean water and air dried under shade at ambient temperature for about a week in the absence of any form of contaminant. It was then pulverized mechanically (using mortar and pestle) to form a fine powder. After pulverization the powdered sample was stored in an airtight container, in cool and dry place away from light and was later subjected to ethanol extraction (Rohit, 2015).

### 2.5 Extraction Process

About 200 g of the powdered leaf sample of *Naucleadiderrichii* was percolated with 3.0 Litre of absolute ethanol with shaking at regular interval for one week, after which the extract was separated from the debris by filtration. The filtrate was then concentrated

using a rotary vapour (R110 at 40°C), coded Nd-CETE (crude ethanol leaf-extract), weighed and kept in a cool and dry place away from any form of contaminant (Shovonet *al.*, 2016).

## 2.6 Maceration of Crude (Nd-CETE) Extract

About ten gram (10 g) of the crude ethanol leaf-extract (Nd-CETE) was then macerated with three different solvents based on polarity gradient (petroleum ether, chloroform and ethyl acetate) in parts, to obtain three different fractions, coded Nd-PETF, Nd-CHLF and Nd-EAF. Each fraction obtained was weighed and concentrated at room temperature.

## 2.7 In Vitro Cytotoxicity Evaluation Using *Artemia Salina* (Brine shrimps)

### 2.7.1 Hatching of *Artemia Salina* Eggs

*Artemia Salina* eggs (Brine shrimps eggs) obtained from Professor O.A. Adoum, Department of Pure and Industrial Chemistry, Bayero University, Kano, Nigeria, was used as the test organism. Natural sea water (100 mL) was taken in beaker (500 mL) and 6.4 g of *Artemia Salina* eggs were introduced into the content. The eggs started hatching after 48 hours in the presence of light and the content was allowed to stand for about 72 hours for complete hatching and then development into matured nauplii (Larvae). This process is carried out under cross ventilation in order to ensure constant supply of oxygen and the nauplii were taken for this assay (Apuaboet *al.*, 2013).

### 2.8 Preparation of Test Sample Solution

About 20 mg of the crude ethanol leaf-extract (Nd-CETE) and each of those of the three fractions (Nd-PETF, Nd-CHLF and Nd-EAF) were dissolved in 2 mL of methanol to give 20,000 µg per 2 mL stock solution. Series of solutions of lower concentrations used for the assay were obtained using two-fold serial dilution to give 1000, 100 and 10 µg/mL, since the volume of the sea water to be used is 5 mL (McLaughlin *et al.*, 1998 and Otanget *al.*, 2013).

### 2.9 Preparation of Control Groups

Control groups were used in cytotoxicity assay to validate the test method and ensure the results obtained were only due to activity of the test agents and the effects of other possible factors are nullified. Positive and Negative control groups were used in this assay.

Positive control in cytotoxicity assay is widely accepted cytotoxic agent and the results of the test agent obtained are compared with the results obtained for the positive control.

In the present study potassium dichromate as a well-known cytotoxic agent is used as a positive control and was evaluated at same concentration

$$\% \text{Mortality} = \frac{\text{Total nauplii} - \text{Living nauplii}}{\text{Total nauplii}} \times \frac{100}{1} \rightarrow (1)$$

(10, 100 and 1000 µg/mL) as that of the test solutions. Uncontaminated sea water was used as a negative control.

## 2.10 Cytotoxicity Assay Procedure

The standard solutions of the crude ethanol leaf-extract (Nd-CETE) and each of those of the three fractions (Nd-PETF, Nd-CHLF and Nd-EAF) were impregnated into dry and clean glass tubes in three (3) replicates of each concentration and the set up was allowed to stand for 24 hours away from any form of contaminant for the diluting solvent to evaporate. After evaporation, few drops of DMSO were introduced into each glass tubes and 2.5 mL sea water was added to make the content ready for the application of Shrimp nauplii (Otanget *al.*, 2013 and Asaduzzaman *et al.*, 2015).

With the help of Pasteur pipette, ten (10) living and healthy nauplii were introduced into each of the glass tube containing the test sample solution and the two control solutions up to 5 mL pre-marked points on the glass tube. For convenient application, a magnifying glass was used for counting and precautions were taken to avoid taking shrimp cyst into the glass tube (Apuaboet *al.*, 2013). After 24 hours incubation, same magnifying glass was used for convenient counting of the shrimp nauplii that survived in each glass tube and recorded. From this data, the percentage mortality of shrimp nauplii was calculated for each concentration using the equation 1 below (Jeremiah *et al.*, 2017)

### 2.11 Extraction and Fractionation of *Naucleadiderrichii* Leaf Extract

The crude ethanol leaf-extract of the plant, *Naucleadiderrichii* yielded 13.5 g (6.7%), while the yield of the macerated fractions can be seen in the Table 1.

### 3.0 RESULTS AND DISCUSSION

#### 3.1 Toxicity Test Result

Brine shrimp cytotoxicity test is considered as a preliminary assessment of toxicity. This assay

determines lethal concentration of active compounds such as heavy metals, pesticides and medicines in brine medium (Solis *et al.*, 1993 and Jaki *et al.*, 1999) and has been

employed to determine toxicity of various active compounds because it is reliable, rapid and very convenient to carry out (Otanget *al.*, 2013; Lahti *et al.*, 1995; Martinez *et al.*, 1999). The percentage mortality of *Artemiasalina* incubated in extract and fractions of *Naucleadiderrichi* and controls (potassium dichromate and Sea water) are shown in Table 2.

**Table 1: Weight, Color and Texture of fractions of *Naucleadiderrichii***

| Fractions | Weight(g) | % Yield | Color        | Texture |
|-----------|-----------|---------|--------------|---------|
| Nd-PETF   | 1.22      | 12.20   | Golden green | Sticky  |
| Nd-CHLF   | 0.5       | 5.00    | Dark green   | Sticky  |
| Nd-EAF    | 0.27      | 2.70    | Dark brown   | Sticky  |

Nd=*Naucleadiderrichii*, PETF= Petroleum Ether Fraction, CHLF=Chloroform Fraction and EAF=Ethyl Acetate Fraction

**Table 2: Brine Shrimp Lethality Test Result for Crude Extract and Fractions**

| Extract/Fractions   | Conc. (µg/mL) | Number of Nauplii used | Nauplii survived After 24 hrs | Percentage (%) mortality | LC <sub>50</sub> (µg/mL) |
|---|---------------|------------------------|-------------------------------|--------------------------|--------------------------|
| Nd-CETE   | 10            | 10                     | 10                            | 0                        | 342.908                  |
|   | 100           | 10                     | 7                             | 30                       |                          |
|   | 1000          | 10                     | 4                             | 60                       |                          |
| Nd-PETF   | 10            | 10                     | 10                            | 0                        | 3239.098                 |
|   | 100           | 10                     | 9                             | 10                       |                          |
|   | 1000          | 10                     | 7                             | 30                       |                          |
| Nd-CHLF   | 10            | 10                     | 10                            | 0                        | 1574.609                 |
|   | 100           | 10                     | 9                             | 10                       |                          |
|   | 1000          | 10                     | 6                             | 40                       |                          |
| Nd-EAF  | 10            | 10                     | 10                            | 0                        | 578.085                  |
|   | 100           | 10                     | 8                             | 20                       |                          |
|   | 1000          | 10                     | 4                             | 60                       |                          |
| Positive control (K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> ) | 10            | 10                     | 0                             | 100                      | 3.481                    |
|   | 100           | 10                     | 0                             | 100                      |                          |
|   | 1000          | 10                     | 0                             | 100                      |                          |
| Sea water (-ve)   | -----         | 10                     | 10                            | 0                        | 0                        |

Nd=*Naucleadiderrichii*, CETE=Crude Ethanol Extract, PETF= Petroleum Ether Fraction, CHLF=Chloroform Fraction and EAF=Ethyl Acetate Fraction

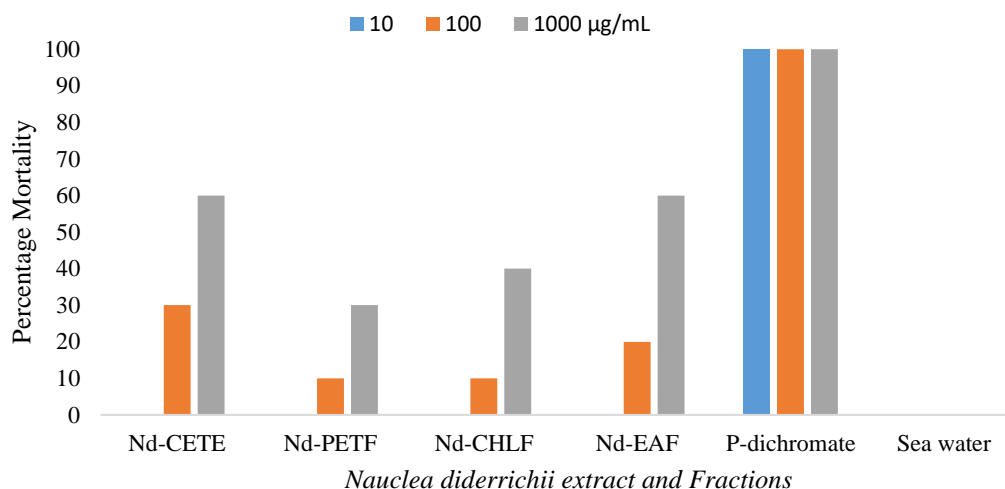
There was high mortality of *Artemiasalina* (brine shrimp nauplii) incubated in both the crude ethanol extract and ethyl acetate fraction, whereas the nauplii incubated with potassium dichromate had a significantly greater mortality when compared to all the extract, fractions and sea water. The ethanol extract, ethyl acetate fraction, chloroform fraction, n-hexane fraction and

seawater showed a mortality of 30 and 60%, 20 and 60%, 10 and 40%, 20 and 60% and 0% at 100 and 1000 µg/mL respectively.

The degree of mortality of nauplii was in a concentration-dependent fashion. The highest mortality was observed in crude ethanol extract and ethyl acetate fraction at 1000 µg/mL compared to potassium

dichromate which showed maximum mortality (100%) at all concentrations. The effect of different concentrations of the plant extract,

fractions and control on the mortality of brine shrimp nauplii is shown in Figure 1.



**Fig. 1: Percentage mortality of *Artemiasalina* incubated at different concentrations of the plant extract, Fractions and control**

According to Meyer *et al.* (1982) and Bastos *et al.* (2009) the brine shrimp lethality were interpreted in accordance to the criterion by Meyer toxicity index that  $LC_{50}$  values  $>1000$  µg/mL are considered non-toxic,  $LC_{50}$  values  $>500$  µg/mL but not  $>1000$  µg/mL are considered to have weak toxicity, while those having  $LC_{50}$  values  $<500$  µg/mL are considered toxic. The  $LC_{50}$  values were calculated as 342.908, 3239.098, 1574.609, 578.085 and 3.481 µg/mL for ethanol crude extract, petroleum ether fraction, chloroform fraction, ethyl acetate fraction and potassium dichromate respectively (Table 1).

The results showed significant activity in both the Crude ethanol leaf-extract and ethyl acetate fraction at 100 and 1000 µg/mL respectively. The crude ethanol leaf-extract (Nd-CETE) and three different fractions (Nd-PETF, Nd-CHLF and Nd-EAF) produced concentration dependent increment in percent mortality of Brine Shrimp nauplii indicating the presence of cytotoxic agents in these extract and fractions (Apuabo *et al.*, 2013). The genus *Nauclea* contains several species, such as *Nauclea latifolia*,

*Nauclea orientalis* and *Nauclea diderrichii*, for which the activity of crude extracts has previously been reported (Lamidi *et al.*, 1996; Benoit-Vicalet *et al.*, 1998; Traore *et al.*, 2000; Traore-Keita *et al.*, 2000; Zirihiet *et al.*, 2005). This activity has been attributed either to

saponins (Lamidi *et al.*, 1996) or numerous alkaloids found in this genus (Phillipson 1982; Abreu and Pereira 2001; Azas *et al.*, 2002). These toxicity test results from this study could be employed as promising alternative in the treatment and management of tumors as brine shrimp lethality test now serves as an indicator for the preliminary screening of bioactivity including for anticancer (Artantiet *et al.*, 2012).

#### 4.0 CONCLUSION

The findings of present study concluded that *Nauclea diderrichii* leaf extract possesses cytotoxic behavior, suggesting the presence of potential bioactive chemical component. The study uncovered that ethanol crude leaf extract is highly toxic ( $LC_{50} = 342.908$  µg/mL) that of ethyl acetate fraction is moderately or weakly toxic ( $LC_{50} = 578.085$  µg/mL) and those of petroleum ether fraction ( $LC_{50} = 3239.098$  µg/mL) and chloroform fraction ( $LC_{50} = 1574.609$  µg/mL) are non-toxic. This toxicity results suggested that ethanol crude leaf extract and some fractions of *Nauclea diderrichii* contain highly active phytochemical ingredients which validates the primitive use of the plant as a curative agent for various infectious diseases. Haudecoeur *et al.*, (2018) suggested that, the sustainability of the plant, *Nauclea diderrichii*, should be kept in mind to adapt local uses and preparation modes of traditional remedies.

Further studies involving phytochemical screening, isolation, characterization and *in vivo* cell lines cytotoxicity test is recommended to substantiate these findings, elucidate and validate stability and develop mechanism of action of the pure active compounds from the plant, *Naucleadiderrichi*.

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