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ANTIOXIDANT AND CYTOTOXICITY STUDIES OF *ELEUSINE INDICA* (LINN.) GAERTN. ON BRINE SHRIMP

[1]Senjobi C.T., [1]Olusesan P. P., Lawal O. I., Bamigboye S.O., [1]Jimoh M.O., and [2]Ettu A.O.
[1]Plant Science Department, Olabisi Onabanjo university, Ago-Iwoye, Ogun State, Nigeria.
[2]Sikiru Adetona College of Education, Science and Technology, Omu-Ajose, Ogun State, Nigeria
***Correspondence Author: comfort.senjobi@oouagoiwoye.edu.ng, +2347033439472**
Orchid ID: 0000-0002-9915-9702

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ABSTRACT

Cancer is currently one of the most dangerous diseases reported all over the world; there are numerous researches ongoing to tackle the effects of chemotherapy on diagnosed patients. Hence, this study aimed at evaluating the antioxidant and cytotoxic potentials of Eleusine indica on Brine shrimp. Fresh E. indica was air dried, pulverized, macerated in absolute methanol at room temperature for 72 hours, and concentrated. Phytochemical screening of the powdered sample was done using standard protocols. The crude extract was further partitioned into different fractions (n-hexane, aqueous, ethyl acetate), screened for their cytotoxic and anti-oxidant activities using brine shrimp lethality assay and 2,2-Diphenyl-1-picrylhydrazyl radical scavenging method respectively. For the anti-oxidant test, gallic acid and catechol were used as control while cyclophosphamide was used as the control for cytotoxicity test. Preliminary phytochemical assay revealed the presence of alkaloids and anthraquinones while saponins and cardiac glycosides were absent. High anti-oxidant activity was recorded with the lethal concentration of aqueous extract E. indica (129% LC_{50}) followed by the crude extract (128.7% LC_{50}) when compare to gallic acid (0.95% LC_{50}) and catechol (1.70% LC_{50}). The crude extract showed high activity against brine shrimp cell. The n-hexane fraction (12.08 LC_{50} µg/mL) showed the highest cytotoxic activity followed by the aqueous fraction (7.72 LC_{50} µg/mL). This study revealed the potent antioxidant and cytotoxic potentials of E. indica and also justified its use in traditional medicine. Further investigation should be carried out on the isolation and characterization of the bioactive compounds responsible for these activities.

Keywords: Antioxidant**,** catechol, cyclophosphamide, cytotoxicity, *Eleusine indica*, gallic acid

INTRODUCTION

For decades, the molecular basis by which normal cells transform into cancerous cells has been the subject of research in the biomedical sciences. Despite this effort, finding cures or long-term therapeutic approaches for metastatic cancer is just as difficult as it was more than 40 years ago when President Richard Nixon declared a war on cancer Seyfried and Shelton, 2010). Cancerous cells undergo cellular division, giving rise to strong and hard tumors or a proliferation of aberrant cells in the blood. Cancer is caused by more than one mutation,

in actuality; a lot of changes to specific sets of genes are usually required before a cell becomes a cancerous cell (Wong and Yu, 2019). Different strategies have been developed to impede the growth and spread of cancer cells. However, the majority of patients receive a mixture of therapies like surgery, chemotherapy, hormone therapy, photodynamic therapy and natural product medication (Akulapalli, 2009). Different chemotherapy medications are used to treat cancer, but the problem of selective toxicities and serious side effects continues. As a result, novel anticancer medication leads derived from natural products are required.

For ages, medicinal plants growing in the tropical areas of Sub-Saharan Africa have been widely used as herbal treatments in local communities (Moyo *et al*., 2015). Nigeria is endowed with diverse medicinal plants, and their therapeutic potentials have been established by various studies (Ogbole *et al.*, 2017). Numerous people in developing countries including Nigeria, particularly those living in rural regions, rely primarily traditional medicine. Interestingly, multiple studies have established traditional medical practitioners' (TMPs') indigenous expertise in the treatment of different malignancies across Nigeria's geopolitical zones (WHO, 2005; Ngulde *et al.*, 2015). There is about one herbalist for every 500 patients in this region (WHO traditional medicine plan 2014-2023) but there is one physician for every 6,700 patients (WHO world health statistics 2010) indicating that traditional herbalists outnumber physicians. As a result, identifying effective plant-derived anti-cancer agents is critical for this group. Despite the fact that the cytotoxicity of 8.7 million plants has been studied (Ogbole *et al.*, 2015), there is yet to be a breakthrough in anti-cancer research. Hence, this study was conducted to provide some justifications to the anti-oxidant and cytotoxic relevance of local plants used in cancer care with the goal of discovering novel anti-cancer drugs.

The plant, *E. indica* often known as the goose grass or wire weed, a member of the Poaceae family, native of West Africa and Asia, also grown in Nigeria, where it is known in Yoruba language as ‘Gbegi’, with the majority of this species being perennial or annual is considered in this study (Redden *et al.*, 2015) Plate 1.

Plate 1: *Eleusine indica* (Linn.) Gaertn. (location: Lapade, Eruwa Road, Oyo state, Nigeria)

Gbadamosi and Otobi (2014) reported that *E. indica* supplies pregnant women with their nutritional needs reducing malnutrition. It also has diuretic, depurative, anti-helminthic, febrifuge and laxative properties (Ettebong *et al.*, 2012). Further to being widely applied in traditional and herbal medicines, the weed's safety in terms of administration was also supported by plants toxicological research. So far, report has shown that there aren't many phytochemicals isolated from *E. indica*, although, the orientin, vitexin, isovitexin, saponarin, tricin, isoorientin, violanthin, and lucenin flavonoid patterns of the *Eleusine* genus were first recognized in 1978 (Devi *et al*., 2014). Only few phytochemicals such as the flavonoids; schaftoside, vitexin, and isovitexin, as well as steroidal glucosides, 3-o-d-glucopyranosyl-sitosterol and its 6'-o-palmitoyl derivatives, have been identified

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from the plant (Desai, 2017; Iqbal and Gnanaraj, 2012). Moreover, recent metabolite fingerprinting and profiling have characterized many more metabolites and amino acids, as well as two new phytochemicals, p-coumaric acid and isoschaftoside (Peñaloza *et al.*, 2018).

MATERIALS AND METHODS

Materials

Cotton-wool, filter papers, micropipette, hand gloves, nose-mask N95, aluminum foil, capillary tubes, droppers, foil paper, dichloromethane (DCM), methanol, N-hexane, ethyl acetate, distilled water, normal saline.

Collection and authentication of the selected plant

Eleusine indica was harvested on July 28, 2022, at Lapade estate in Oyo state. Dr. Odewo of the Forest Research Institute of Nigeria authenticated the plant at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, Oyo State, Nigeria, where the Forest Herbarium Index (FHI) number was obtained as 113559.

Preparation of plant materials

To avoid contamination, plant material was properly washed and air dried for three weeks. it was further dried in the oven at 60^0C before pulverization and kept in clean glass jar until it was ready for use.

Phytochemical screening

Phytochemical screening was done on the pulverize sample of *E. indica* to check for the presence or absence of metabolites. The preliminary phytochemical procedures were carried out according to the standard procedures employed by (Ayoola *et al.*, 2008; Adefuye, 2013).

Test for alkaloids

Powdered sample of *E. indica* (1g) was placed in a clean test tube, followed by 10ml of 10% hydrochloric acid (HCL). It was then cooked for 10 minutes on the Bunsen burner prior to being filtered. Ammonia (NH_3) was used to alter the pH to around 6-7. In separate test tubes, 0.5mL of each filtrate was placed, and 2-3 drops of Dragendoff, Mayer, and Wagner's reagent were added and mixed together. A reddish brown or cream precipitate indicates the presence of alkaloids

Test for tannins

Dried sample of *E. indica* (1g) was separately boiled with 20mL of distilled water for 50mins filtered while hot and allowed to cool. After diluting the filtrates to 5mL with distilled water, 2-4 drops of ferric chloride (0.1%) were added to the final solution. Tannins are indicated by a blue-black, green, or blue-green

Test for saponins

Dried powdered sample of *E. indica* (1g) was separately boiled with 10mL of distilled water for about 10mins and filtered immediately after boiling. On cooling, the filtrate was tested for;

a. Frothing: the production of froth signified a successful outcome.

b. Emulsifying characteristics: observed after adding 2 drops of olive oil to each test tube containing dried material.

Test for anthraquinones

Dried sample of *E. indica* (1g) was placed in a dry test tube and 5ml of chloroform was added and shaken for 5mins. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. The presence of a rose-pink color in the aqueous layer indicated the presence of anthraquinone.

Test for flavoniods

Dried sample of *E. indica* (1g) was boiled separately with 10mL of distilled water for about 10mins and filtered while hot. After cooling, 1mL of each filtrate was dissolved in 80% alcohol and boiled on a water bath, while magnesium and concentrated H_2SO_4 produced a red color, indicating the presence of flavonoid.

Plant extraction

Pulverized *E. Indica* (150 grams) was extracted with 5 liters of methanol for 72 hours, where they were shaken from time to time for about 48 hours. Thereafter, the sample was filtered using a cotton-wool, the filtrate was then concentrated to dryness on the water bath. The percentage yield of each extract was then stored in petri dish. The extraction yield is a metric for how effectively the solvent extracts of a particular component from the starting material. Then the crude extract yield of the concentrated *E. indica* was taken, and calculated using the below formula:

% = (weight of crude extract/ weight of the sample) × 100

Solvent-solvent partitioning of the extract of *Eleusine indica*

The crude extract of *E. indica* was dissolved in aqueous methanol (methanol and water in ratio of 3:1) and was poured in the separating funnel, and allowed to settle hence creating the first layer of the partition. Followed by adding equal volume of hexane and this was mixed gently with the aqueous-methanol and allowed to settle down. With the aid of a bright light source (touch light), the hexane fraction was identified and pulled out gently form the separating funnel. This procedure was repeated till the solvent became clear. A more polar solvent ethyl acetate was used, the same process as describe for the hexane fraction was repeated to get the ethyl acetate fraction and the aqueous fraction. The fractions collected were poured in a ceramic bowl and air-dried.

Brine shrimp lethality bioassay

The brine shrimp lethality bioassay was used to determine the cytotoxicity of the extracts. Artemia salina shrimps were produced using Artemia salina eggs in a container filled with brine solution, which is made by dissolving 38 g of sea salt in 1000 mL of distilled water and adjusting the pH to 8.5 using 1ml NaOH. The process took 48 hours with continuous aeration. Live-shrimps were gathered and utilized in the test (Rafshanjani *et al.,* 2014). In each test tube, 4.5 mL of the brine solution were added. The extract of *E. indica* was diluted appropriately in accordance with concentrations. The shrimp test tubes received 0.5 mL of the diluted solution of *E. indica*. Each test tube had ten live shrimp introduced to it using a glass dropper tube. After 24 hours, the surviving (larvae) shrimps were counted, and the lethality concentration LC50 determined.

% death = (number of dead nauplii /(number of dead nauplii + number of live nauplii)) × 100

DPPH Radical Scavenging Activity

The free radical scavenging activity of the crude extracts of *E. indica* and it partitioned fractions was evaluated using the method described by Sonibare and Adediran (2017) with slight modifications. The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability with gallic acid and catechol as positive control. One mL (1 mL) of methanol solution of test samples and standard (gallic acid and catechol) at different concentration (100, 50, 25, 12.5, µg/mL) were mixed separately with 3 mL (0.004 %) of freshly prepared 2, 2-diphenyl-1-picryl-hydrazylhydrate (DPPH-Sigma Aldrich). For the blank, 1 mL of methanol 3 mL of DPPH was mixed with it. The reaction mixtures were incubated at room temperature and allowed to react for 30 minutes in the dark. After 30 minutes, the absorbance was measured at 517 nm using UV-3100PC Spectrophotometer (Spectrumlab 725S) and converted into percentage of antioxidant activity, results were recorded in triplicates. The degree of decolourisation of DDPH from purple to yellow indicated the scavenging efficiency of the extract (Adediran and Sonibare, 2017). The concentration of sample required to scavenge 50% of the DPPH free radical (IC50) was determined from a calibration curve using the equation of the

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graph. The percentage of inhibition of DPPH (%) was calculated as follows:

% inhibition = (absorbance of control – absorbance of test sample)/absorbance of control) × 100

The antioxidant activity of each sample was expressed in terms of IC50.

RESULTS

Extraction

The percentage yields of the methanol extract from the aerial part of *E. indica* and fractions of liquid-liquid chromatography from the extract are shown in Table 1 below. The methanol extract of *E. indica* has a percentage yield of 8 %, the percentage yield of the crude extract was also calculated based on fractions. Among the four fractions (hexane, Aqueous, ethylacetate and methanol) obtained, the highest percentage yield was recorded from ethyl acetate and aqueous extracts (25%), followed by *n*-Hexane yield (16.7%) and the least yield (8%) was obtained from Methanol fraction of *E. indica* extract.

Table 1: Yields of crude of whole plant of *Eleusine indica* and its fractions in different solvents

Eleusine indica	Solvent used	Weight of extract (g)	Yield (%)	Colour of extract
Whole plant	Methanol	12	8	Deep green
Whole plant	Aqueous	3	25	Brown
Whole plant	Ethyl acetate	3	25	Black
Whole plant	Hexane	2	16.7	Deep green

Preliminary phytochemical screening

As indicated in Table 2, the preliminary phytochemical screening of the powdered extract of *E. indica* showed the presence of alkaloids, flavonoids, tannins and anthraquinones, while saponins and cardiac glycosides were absent.

Table 2: Phytochemical screening of the pulverize whole plant of *Eleusine indica*

Secondary metabolites	Inference
Saponins	-
Tannins	+
Alkaloids	++
Anthraquinones	++
Polyphenols (flavonoids)	+
Cardiac glycosides	-

Keys: - (absent), + (fairly present), ++ (moderately present), +++ (abundantly present).

Cytotoxicity Activity

The cytotoxicity activities of the crude extracts, the partitioned fractions of *E. indica* and the control, cyclophosphamide on *Artemia salina* (brine shrimp) are shown in Table 3. The death of brine shrimps is expressed as mean percentage death ± standard error of mean. It was observed that the percentage death of *Artemia salina* on the plant extract were dose dependent, with 1000 µg/mL dose having the highest activity for all the extracts and the fractions while 1.95 µg/mL had the lowest activity across all the plant extract tested. This suggests that with increased dose, there was a notable increase in the activity of the extracts. While Table 4 showed the lethal concentration at 50, this also showed that n-Hexane extract has the highest lethal concentration at 50% (12.08 µg/mL) with ethyl acetate having the lowest at 0.13 µg/mL.

Table 3: The percentage death of *Artemia salina* (brine shrimps) using crude extract and fractions of *Eleusine indica*

Conc (µg/mL)	Crude extract	n-Hexane	Ethyl acetate	Aqueous extract	Cyclophosphamide
1000	100±0.00	100±0.00	100±0.00	100±0.00	80.0±0.00
500	100±0.00	100±0.00	100±0.00	100±0.00	70.00±0.00
250	100±0.00	100±0.00	100±0.00	100±0.00	63.6±0.03
125	100±0.00	100±0.00	100±0.00	100±0.00	56.6±0.03
62.5	100±0.00	83.33±0.16	100±0.00	100±0.00	36.67±0.06
31.2	100±0.00	72.22±0.05	100±0.00	94.44±0.05	3.33±0.03
15.63	77.7±0.00	61.11±0.05	100±0.00	66.66±0.11	1.55±0.03
7.80	44.4±0.11	44.44±0.11	100±0.00	44.44±0.00	0.55±0.00
3.90	38.80±0.05	16.66±0.05	95.5±0.00	33.33±0.00	0.00±0.00
1.95	38.80±0.05	0.55±0.05	75.5±0.00	16.66±0.05	0.00±0.00

Table 4: Lethal concentration at 50 (LC_{50}) of crude extract and fractions of *Eleusine indica*

Samples	LC_{50} µg/mL
Crude	5.26±0.07
n-Hexane	12.08±0.03
Ethyl acetate	0.13±0.03
Aqueous	7.72±0.04
Cyclophosphamide	61.82±0.02

Antioxidant Activity

The dose dependent 2,2-dipheyl-1-picrylhdrazyl (DPPH) free radical scavenging activity of both the crude and the partitioned fractions of *E. indica* are shown in Table 5. It was observed that the free radical scavenging activity of the plant extracts were dose dependent, with 100 µg/mL dose having the highest activity for all the extracts and the fractions while 12.5 µg/mL had the lowest activity across all the plant extracts tested. This suggests that with an increase in dose, there was a notable increase in the activity of the extracts. The LC50 inhibitory concentration scavenging of crude and fractions of *Eleusine indica* when compared with Gallic acid and Catechol had higher values Table 6.

Table 5: Percentage (%) scavenging activity of crude extract and fractions of *Eleusine indica*

Conc. (µg/mL)	Crude extract	Hexane	ethyl acetate	Aqueous	gallic acid	catechol
100	25.96±0.00	43.91±0.03	27.88±0.00	26.56±0.00	82.53±0.00	80.36±0.00
50	5.84±0.00	18.66±0.00	13.78±0.00	12.82±0.00	81.57±0.00	79.80±0.00
25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	79.08±0.00	77.88±0.00
12.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	79.72±0.00	78.76±0.00

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Table 6: 50% inhibitory concentration scavenging of crude and fractions of *Eleusine indica*

Samples	LC50
Crude	128.7±0.00
Hexane	105.7±0.00
Ethyl acetate	127.8±0.00
Aqueous	129.6±0.00
Gallic acid	0.95±0.00
Catechol	1.70±0.00

DISCUSSION

Bioactive compounds are obtained from biomass materials through extraction yield. The objective of extraction is to maximize the amount of target compounds and to obtain the highest biological activities of these extracts (Chang *et al.,* 2002). The yield of the extract of *E. indica* at room temperature was higher for ethyl acetate and aqueous extracts (25 w/w) than *n*-hexane fraction (16.88 % w/w) as shown in Table 1, this is due to the presence of methanol which as a solvent is known for giving high extractive yield because of its polarity as it can extract both hydrophilic and lipophilic molecules from plant parts. Likewise, it is known to have good cell penetration (Daud *et al.,* 2022).

The result of the phytochemical screening conducted on the powdered sample of *E. indica* showed that it contains tannins, flavonoids, alkaloids, and anthraquinones while cardiac glycosides and saponins were absent, this was in agreement with Alaekwe *et al*. (2015) who reported the absence of Saponins in the crude extract. The phytochemical evaluation corroborated the discovery of Rashida and Nisha (2022) and Okokon *et al*. (2010), that flavonoids, tannins, alkaloids, and anthraquinones are present in *E. indica* with dart variation which may be due to the different extracting medium (Morah and Otuk 2015; Akouavi *et al*., 2021).

The cytotoxic activity of *E. indica* when compared to the standard (cyclophosphamide) was high as shown in table 5, the lethal concentration at 50 (LC50) of the partitioned fractions and the crude extract showed high cytotoxic activity. The activity exhibited by the n-Hexane extract (12.08 %) was the highest compared to other partitioned fractioned, all extract showed high cytotoxicity against the control (cyclophosphamide). The presence of these phytochemicals could be responsible for the potent cytotoxic and anti-oxidant potentials of *E. indica* (Trease and Evans, 1989; Panche *et al.,* 2016).

The radical scavenging activity (DPPH) of *E. indica* when compared to the standard (Gallic acid and catechol) was low as shown in Table 5 and 6. The inhibitory concentration at 50 (IC_{50}) of the partitioned fractions were lower than the methanol crude extract except the aqueous fraction of *E. indica* which implies more activity than the methanol crude extract. The activity exhibited by the crude extract (105.7µg/mL%) was the highest compare to other partitioned fractioned, all extract showed high activities when compared with the control (gallic acid and catechol). The strong anti-oxidant potential of *E. indica* might be part of the factor associated to its strong tolerance to herbicides (Sunohara *et al.,* 2011).

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