Journal of Medicinal Plants Studies

www.PlantsJournal.com

Ette O Ettebong
Department of Clinical
Pharmacology and Therapeutics,
Faculty of Basic Clinical
Sciences, College of Health
Sciences, University of Uyo,
Nigeria

Peace ME Ubulom
Department of Animal and
Environmental Biology, Faculty
of Science, University of Uyo,
Uyo, Nigeria

Daniel Obot
Department of Clinical
Pharmacology and Therapeutics,
Faculty of Basic Clinical
Sciences, College of Health
Sciences, University of Uyo,
Nigeria

A Systematic review on *Eleucine indica* (L.) Gaertn.): From ethnomedicinal uses to pharmacological activities

Ette O Ettebong, Peace ME Ubulom and Daniel Obot

Abstract
Eleusine indica is a useful medicinal plant that is widely distributed in the tropical and tropical regions of Africa, Asia, and South America. This article is aimed at preparing, providing and enhancing a comprehensive review on ethnomedicinal and pharmacological values of *Eleusine indica* plant. Literature search included articles in databases such as Google Scholar, Hinari, PubMed and Science Direct. General Google search, books and online resources were also used. Keywords such as *Eleusine indica*, ethnomedicinal uses, phytochemistry and pharmacological activities were used. The botanical name *Eleusine indica* was identified using The Plant List database (theplantlist.org). Several chemical compounds have been identified such as phenols, anthrones, coumarins, essential oils, triterpenes, steroids, fatty acids, anthraquinones, anthrones, tannins, flavonoids, alkaloids and coumarins. Two main flavonoids have been isolated from the aerial parts: schaftoside and vitexin. Orientin and Isoorientin and other compounds have also been islolated. From this review, this plant exhibits a large array of pharmacological properties such as antiplasmodial, anti-inflammatory, analgesic, antipyretic, antiviral, anticonvulsant, antimicrobial, antioxidant, antileishmanial, anthelminthic, cytotoxic, antitrypanosomal and antihypertensive properties. This review has provided the scientific justification for some of the ethnomedicinal uses of *E. indica* in the treatment of common diseases that plague humans and depicts that the plant has a promising medicinal potential that should be exploited.

Keywords: *Eleusine indica*, pharmacology, ethnomedicinal uses, extracts, compounds

Introduction

Medicinal plants obtained from the tropics of sub-Saharan Africa have, for ages, been widely used as traditional remedies for local communities. They still continue to provide a significant therapeutic alternative for a large part of the population [1]. In this region, there is approximately one herbalist to 500 patients [2] whereas there is one physician to 6,700 patients [3], which shows that there are more traditional herbalists than there are physicians. The rain forests of tropical Africa south of the Sahara has one of the richest and most diverse ecosystems of the world accounting for about 40% plants species globally despite the fact that it represents only 7-8% of emerged land surface [4, 5]. There are nine species in the genus Eleusine which are mostly found in the subtropical and tropical areas of Africa, Asia, and South America. These species are *Eleusine indica, Eleusine coracana, Eleusine africana, Eleusine intermedia, Eleusine multiflora, Eleusine jaegeri, Eleusine kigeziensis, and Eleusine tristachya*. Most of these species occur in the wild as perennial or annual [6]. *Eleusine indica* belongs to the family Poaceae, formally called Gramineae. It is most probably the ancestor of *Eleusina coracana* [7]. This is the grass family and constitutes the fifth largest family of flowering plants having about 50 tribes, 660 genera and 10,000 species [8]. It is considered native to Africa, temperate and tropical Asia, it is now widely distributed throughout the tropics, extending into the subtropics of North America, Europe and Africa.

Eleucine indica, L. Gaertn (Poaceae) has common names which include: wire grass, goose grass or crowsfoot (English), Dogon (Mali), and Fula – pulanar in Senegal [9, 10]. It is called nkimenang by the Ibibios of South-South Nigeria and 'Jangali Marua' in Hindi [11, 12].

It is an annual plant and can grow to 60 cm in height, slender or moderately robust or sprawling prostrate to 1.2m long. It is considered to be an adventitious species that is probably originally native to Africa but has become a pantropical weed [13, 14, 9]. The stem is usually either white or pale green and is flattened on the lateral side having a few strands of "grainy hair" on the edges.

Corresponding Author:
Ette O Ettebong
Department of Clinical
Pharmacology and Therapeutics,
Faculty of Basic Clinical
Sciences, College of Health
Sciences, University of Uyo,
Nigeria

The leaf sheaths are also flat laterally and the leaf blades are flat or folded and linear-lancoelate as shown in Figure 1 [15, 16]. A single plant of *E. indica* is capable of producing more than 50,000 small seeds, which can disperse easily by wind and water, and through attaching to animal fur and machinery and as a contaminant in soil [17]. Viable seeds may persist in the upper soil for only 2-5 years [18, 19]. *E. indica* can invade natural areas when the habitats are disturbed and the margins of natural forests as well as marshes, grasslands, stream banks and coastal areas. It is commonly found as weed along roads and pavements [20]. *Eleusine indica* is reported to be one of the world's most destructive annual weed species with potent fertility and capable of producing more than 40 000 seeds per plant [21]. *Eleusine indica* is fecund, a very competitive and cosmopolitan species being found across a range of soils and temperatures [22]. It infests a wide range of crops causing major crop yield loss in maize, cotton, sweet potatoes, upland rice, sugarcane and many fruit and vegetable orchards [23, 24].

E. indica is known to be resistant to herbicides [25, 26, 27, 28, 29, 30, 31, 32].

The aim of this review is to update and provide adequate knowledge of the ethnomedicinal uses, the phytochemical composition and the validated pharmacological properties of this plant and thereby opening the pathway for its medicinal exploitation and future research.

Materials and Methods
Literature search included articles from databases such as Google Scholar, Hinari, PubMed and Science Direct. General Google search, books and online resources were also used. The botanical name *Eleusine indica* was identified using The Plant List database (theplantlist.org).

Results and Discussion

A: *Eleucine indica*
(whole plant)

B: *Eleucine indica* in the wild

C: *Eleucine indica* - full grown size with its inflorescence

Fig 1: *Eleucine indica* plant

Ethnomedicinal and Non-medicinal Uses
Decoction of fresh leaves used as antihelmintic, diuretic and for treatment of dysentary [33, 34]. In Sablan, Benguet Province of the Philippines, the leaves decoction is used for treatment of arthritis and kidney problems [35]. The decoction of the leave juice is used in Cameroon after childbirth to expel placenta, treat diarrhea, dysentery, epilepsy, and intestinal occlusion and also by the Ibibios of Sout-South Nigeria in the treatment of malaria related fever, diabetes, stomach disorders and infections [36, 37]. It is also used as remedy for hypertension [38]. The whole plant is diuretic, laxative, and depurative and used in the treatment of influenza, hypertension, and oliguria [39, 15, 40]. The whole plant extract is also used to treat feverish and liver disorders. Decoction from the seeds is used to treat infants suffering from black jaundice [36]. The roots are used as part of herbal mixture to treat uterine prolapse [38]. The roots also are used in the treatment of snake bites [41]. Fresh root is fed to treat gonorrhea by tribal people [42]. The decoction of roots is used for asthma and as pain relief for abdominal muscle strain. It is applied to wounds to stop the bleeding. The plant is also used to treat sprains and dislocation. The grass decoction is used as tonic and to relieve bladder disorders [15, 36]. The dried leaves and stems are burnt and used as repellent against hematophagous insects. The grass, when young and tender, is eaten by cattle, goat, dogs, rats and chicken for abdominal disorder and also used as antipyretic for herbivores [12, 23, 43]. The seed is sometimes used as a famine food as well as in the treatment of liver complaints [44].

Ethnobotany of *E. indica*
Domain:	Eukaryota
Kingdom:	Plantae
Phylum:	Spermatophyta
Subphylum:	Angiospermae
Class:	Monocotyledonae
Order:	Cyperales
Family:	Poaceae
Genus:	Eleusine
Species:	*Eleusine indica*

## Phytochemical Studies
Mineral Analysis
The grass contains dry matter 35.8%, crude protein 12.4% [45], moisture 50.91 %, lipid 7.14%, fibre 27.56% and total carbohydrate 80.19% [46]. Elemental detection analyzed using ICP-QES on a dry weight basis expressed as ppm sample showed Calcium (21240), Potassium (25050), Magnesium (4049), Phosphorus (2375), Boron (24.74), Copper (55.12), Iron (455.0), Manganese (163.8), Molybdenum (13.49), and Zinc (80.23) [47].

## Phytochemical composition of *E. indica*
Phytochemical screening carried out using thin layer chromatography detected the presence of secondary metabolites in hot water extract such as phenols, anthrones and coumarins while essential oils, triterpenes, steroids, cardiac glycosides, fatty acids, anthraquinones, anthrones,

tannins, flavonoids, alkaloids, and coumarins were found in variously in ethanol, methanol, acetone, aqueous, n-hexane and ethyl acetate extracts as shown in Table 1[36, 46, 48, 49]. Two main flavonoids have been isolated from the aerial parts: schaftoside (6-C-β-glucopyranosyl-8-C-α-arabinopyranosylapigenin) and vitexin (8- C-β-glucopyranosylapigenin) based on 1H and 13C NMR spectra as shown in figure 4 [39, 50]. Also, spectral analysis from the aerial parts of *E. indica*, two compounds have been isolated and these are 3-0-beta-D-glucopyranosyl-Beta-sitosterol and 6'-0-pal-mitoyl-3-0-beta-D-glucopyranosyl-beta-sitosterol. It is known that 6'-0-pal-mitoyl-3-0-beta-D-glucopyranosyl-beta-sitosterol is a derivative of 3-0-beta-D-glucopyranosyl-Beta-sitosterol [51]. Further spectral analyses of E. indica yielded two compounds Orientin and Isoorientin. Compounds that have been isolated from the chloroform and methanol extracts were found to be derivatives of Hexadecanoic acid, phosphatidyletholamine and ethanediyl ester. The compounds are 1-[[[(2-aminoethoxy) hydroxyphosphinyl] oxy]methyl]- 1,2- ethanediyl ester and and Hexadecanoic acid for the chloroform and methanol extracts respectively as shown in figure 2 [10, 52].
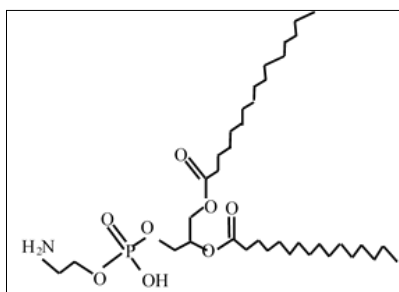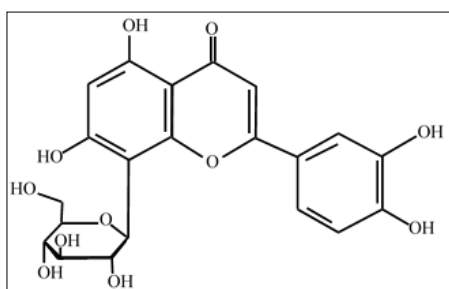
A: Hexadecanoic acid, 1-[[[(2-aminoethoxy) hydroxyphosphinyl] oxy]methl]-1,2-ethanediyl ester. ($C_{37}H_{74}NO_8P$)
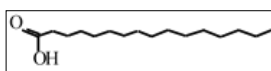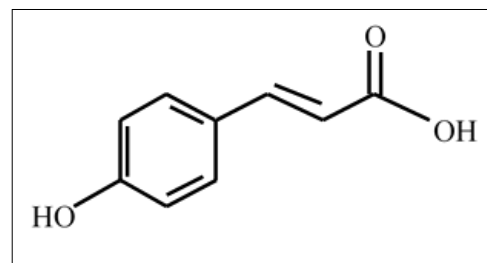
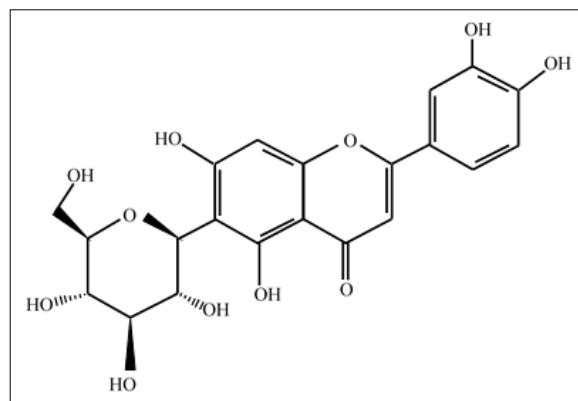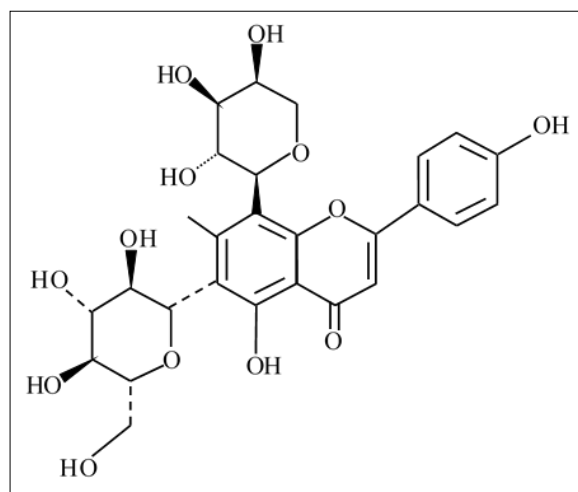**B:** Hexadecanoic acid ($C_{16}H_{32}O_2$)
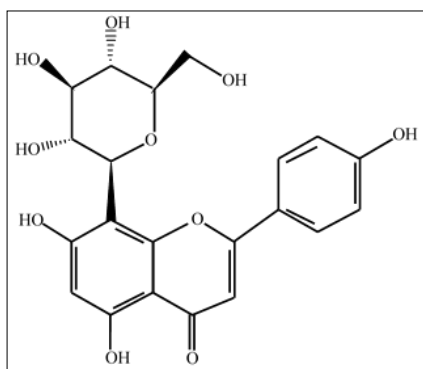
C: Vitexin

D: Orientin

E: p-Coumaric-acid

F: Isoorientin

G: Schaftoside

Fig 2: Compounds isolated and derived from *Eleusine indica*

Pharmacological Properties of *Eleusine indica*
Eleusine indica and its bioactive compounds were found to possess important pharmacological properties such as antiplasmodial, anti-inflammatory, analgesic, antipyretic, anticonvulsant, antimicrobial, antioxidant, antileishmanial, anthelminthic, cytotoxic, antiviral, antitrypanosomal, antihypertensive and toxicological properties.

Antiplasmodial Activities
The antiplasmodial properties of *Eleucine indica* was evaluated *in vivo* using the whole-plant extract and fractions (which included n-hexane, chloroform, ethyl acetate, butanol and aqueous) of *E. indica* against *Plasmodium berghei berghei* infected mice. Extract (200, 400 and 600 mg/kg and) and fractions (400 mg/kg) were screened in suppressive (4-day), prophylactic (repository) and curative tests. Chloroquine (5 mg/kg/d) and pyrimethamine (1.2 mg/kg/d) were used as standard drugs, while distilled water (10 mL/kg/d) served as control.

From these models, the suppressive (4-day) test, showed a dose-dependent decrease in the levels of parasitaemia following administration of the extract compared to control. This decrease was statistically significant ($p < 0.05 - 0.001$). However, the suppressive effect was less when compared with the standard drug chloroquine. The fraction having the highest chemosuppressive effect was ethyl acetate fraction (77.89 %). The degree of parasitaemia suppression was in this order: ethylacetate > n-hexane > aqueous > chloroform > butanol. In the repository (prophylactic) test, the extract showed a dose-dependent decrease in parasitaemia and this decrease was statistically significant ($p < 0.001$) when compared to control. In the Curative Test (Rane Test), there was a progressive dose- and time-dependent reduction in parasitaemia when the extract was tested on established infection. The mean survival times of extract-treated groups of mice were dose-dependently and significantly longer. The extract increased the mean survival time from 12 to 14 days when compared to control. However, when compared to the standard drug, chloroquine, the mean survival time was shorter. The evaluation of the crude extract of *Eleucine indica* and its fractions showed that the plant has a great potential as an antimalarial agent as observed in its suppressive, repository and curative activity against mice infected with *Plasmodium berghei berghei* [11]. The *in vitro* activity of *E. indica* was later screened against chloroquine sensitive *Plasmodium falciparum* strains (Pf 3D7) and Chloroquine resistant *P. falciparum* strain (Pf INDO). It was reported that *E. indica* had IC50, Pf INDO of > 100 μg/ml and IC50, *Pf3D7, of* 85.60±43.23 (μg/ml) which depict moderate activity probably due to the crude nature of the extract [53].

Further antiplasmodial assay of the extract of *E. indica* using P. falciparum D6 (chloroquine-sensitive) and could not go beyond the primary screening because the extract only showed 37 % activity and did not exhibit significant inhibitory activity against the parasite [54].

The observed antiplasmodial activity may be associated with the presence of active compounds such as alkaloids, flavonoids and tannins [55].

Analgesic Properties
The analgesic properties of *E. indica* had been assessed using three pharmacological models: acetic acid-induced writhing, hot-plate test and formalin-induced paw-licking in mice Animals were pretreated with 200 – 600 mg/kg of *E. indica* extract intraperitoneally. Distilled water (10 ml/kg) served as negative control while the standard drug was acetylsalicylic acid (100 mg/kg, i.p.). In the acetic acid-induced writhing, antinociception was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with the extract. The result showed the extract of *E. indica* (200 – 600 mg/kg) dose-dependently reduced acetic acid–induced abdominal constrictions and stretching of the hind limbs. This reduction was significant ($p < 0.001$) relative to control.

In the formalin test, the amount of time each mouse spent licking the injected paw was timed, and was used as indication of pain. The first of the nociceptive response normally peaks 5 min after injection and the second phase 15-30 min after formalin injection, which represent the neurogenic and inflammatory pain response, respectively. The result showed that the animals pretreated with the extract (200 – 600 mg/kg) had a significant ($p < 0.05–0.001$) dose–related reduction in hind paw licking caused by formalin relative to control.

The hot plate test was used to measure the response latencies. The hot plate was kept at 45 ± 1 ˚C throughout these experiments. The mice were placed into a glass beaker of 50 cm diameter on the heated surface, and the time (s) between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30 sec cut-off was used to prevent tissue damage. The result showed that animals pretreated with *E. indica* extract (200–600 mg/kg) depicted a dose – dependent increase in the latency response in the hot plate test. These observed increases in latency response (analgesic effect) were statistically significant ($p < 0.001$) relative to control [56].

Anti-inflammatory Activities
Whole plant decoction is used as anti-inflammatory agent in Nigeria [57]. Investigation of the effect of *E. indica* extract on lung inflammation has been reported. Groups of male BALB/c mice six per group and weighing 25 – 30 g were used in the study. Mice were pretreated intraperitoneally with different doses of *E. indica* or flavonoid-enriched fraction re-suspended in saline and with the flavonoids vitexin and schaftoside re-suspended in 1% DMSO, I h before inhalation of 2.0 mL LPS (0.5 mg/mL) prepared in saline. Another group of mice were pretreated with 10 mg/kg roligram (a selective inhibitor of Phosphodiesterase 4), prepared in saline. Using the same protocol LPS inhalation was done. Three hours following inhalation, the bronchiolar lavage fluids were collected to a final 1.5 mL volume and this was used to assess number of neutrophils by differential counts under light microscope and results expressed as percentage from control. The result shows that intraperitoneal administration of 10 mg/kg of rolipram 1 h before inhalation fully inhibited LPS-induced recruitment of neutrophils. When *E. indica* was subjected to further fractionation, it yielded two major flavonoids, namely, schaftoside (6-C-β-glucopyranosyl-8-C-α-arabinopyranosylapigenin) and vitexin (8-C-β-glucopyranosylapigenin) from spectral analysis. Administration of 400 μg/kg vitexin or scaftoside inhibited 80% and 62 % neutrophil recruitment induced by LPS respectively. Both flavonoids are potential anti-inflammatory compounds as demonstrated by their high ability to inhibit neutrophil migration in mice. The researchers concluded that C-Glycosylflavones from aerial parts of *E. indica* inhibited LPS-induced mouse lung inflammation [39].

It is well known that Lipopolysaccharide (LPS) induces dendritic cell maturation and produces a high level of TNF-α. The anti-inflammatory activity of *Eleucine indica* using TNF-α production by imDC (immature dendritic cells) after culture of DC with different concentration of the plant extract in the presence of LPS. The result indicated that *E. indica* extract dose-dependently inhibited the LPS-induced TNF-α production by imDC. Knowing that in response to phosphoantigen stimulation, γδ T cells produce high levels of cytokines including TNF-α and begin to proliferate. Also evaluated was the effect of E. indica extract on both the cytokine production and proliferation of γδ T lymphocytes. Similarly, *E. indica* extract dose-dependently inhibited IPP-induced TNF-α production ($p < 0.05$). The above results depict anti-inflammatory activity. However, when PBMCs (peripheral blood mononuclear cells) of healthy donors were incubated for 10 days with IPP (isopentenyl pyrophosphate) and different concentrations of extract in the presence of IL-2, the extract at 5 μg did not inhibit γδ T cells proliferation [14].

Assessment of the effect of *E. indica* extract on acute inflammation in mice has been reported using three models:

Cararrgeenin-induced hindpaw oedema, egg albumin-induced inflammation and xylene-induced ear oedema in mice. The results showed that the extract had good anti-inflammatory effect against acute inflammation by suppressing dose – dependently the increase in the mouse paw oedema caused by carrageenin. This inhibition was statistically significant ($p <$ 0.05- 0.001) relative to control. The inhibition caused by the extract (600mg/kg) was comparable to that of the acetyl salicylic acid and was maximal after 5 hours of administration of phlogistic agent. In egg albumin-induced oedema test, the results showed that the extract caused inhibition of egg albumin-induced oedema in mice over a period of 5h. These effects were dose- and time-dependent and statistically significant ($p < 0.01 - 0.001$) relative to control. In xylene – induced ear oedema in mice, the result indicated a dose-dependent inhibition of mice ear oedema by the extract. This inhibition was statistically significant ($p < 0.001$) relative to control.The degree of inhibition was favourably comparable with the standard drug Dexamethasone [56].

Antipyretic Activities
The antipyretic properties of *Eleusine indica* have been investigated. Basal rectal temperatures of adult albino rats of both sexes were recorded and the animals fasted for 24 h but allowed access to water ad libitum. They were then treated with DNP (10 mg/kg) and amphetamine (5 mg/kg) intraperitoneally. Within 30 min following the administration of amphetamine, animals with increased temperature of 1∘C were selected and randomized into five groups of six animals each. Group 1 received 10 ml/kg of distilled water orally. Group's 2 - 4 animals were administered 200 – 600 mg/kg of the extract intraperitoneally respectively. Group 5 animals received 100 mg/kg of acetyl salicylic acid orally. Yeast-induced pyrexia was achieved using 10 ml/kg of Brewer's yeast suspension injected subcutaneously in the back below the neck. Rectal temperatures were then obtained at 0.5 h and thereafter hourly for 5h.

In yeast – induced pyrexia in rats, subcutaneous injection of yeast suspension markedly increased the rectal temperature 8 h following administration. The extract decreased the rectal temperature in a dose- and time-dependent manner. This decrease was statistically significant ($p < 0.01 - 0.001$). In Amphetamine – induced pyrexia in rats, the extract showed significant ($p < 0.05 - 0.001$) and dose-dependent reduction in the elevated body temperature. The highest dose of the extract (600 mg/kg) compared well with acetyl salicylic acid. In Dinitrophenol (DNP) – induced pyrexia in rats, the results showed a dose- and time-related decrease in temperature compared to control. This decrease was statistically significant ($p < 0.05 - 0.001$) [58]. Pyrexia results from infectious agents or damaged tissues initiating the increase production of pro-inflammatory mediator cytokines such as interleukin 1β, β, α and TNF-α which enhance the formation of prostaglandin E2 (PGE2) in the hypothalamus and the prostaglandin then acts on the hypothalamus to elevate the body temperature [58]. Flavonoids, which are present in this plant, are known to be prominent inhibitors of Cyclooxygenase or Lipooxygenase [60]. Therefore, it may be assumed that the extract of E. indica may have acted as a cyclooxygenase-2 (Cox-2) inhibitor through the inhibition of PGE_2 production in the hypothalamus or by enhancing the production of endogenous antipyretic substances in the body such as vasopressin and arginine, or by vasodilatation of superficial blood vessels with resultant increased dissipation of heat after resetting the hypothalamic thermostat. Several

studies have observed that the antipyretic properties of plants are traceable to the presence of steroids, tannins, triterpenoids, flavonoids and coumarin glycosides [61].

Antioxidant Activities
E. indica extracts have been evaluated for antioxidant properties. Two extracts, the hydrolysed (total antioxidant) and non-hydrolysed (free antioxidant) were used to assess the total phenolic content at 750nm using Folin-Ciocalteu reagent diluted 10 times before use with catechin as standard and optical density read aafter 20 min of incubation. DPPH free radical scavenging activity was also examined using the two extracts as well as Ferric reducing antioxidant power (FRAP). When the results of the Free total antioxidant capacity (mg of catechin equivalent/g of dry weight) by FRAP, Folin and DPPH methods were analyzed, DPPH scavenging activity shows FRAP free antioxidant value of 1.94 ± 0.48 mg/g, FRAP total antioxidant, 18.96 ± 1.15 mg/g; Folin free Polyphenol, 0.46 ± 0.10 mg/g; Folin total Polyphenol 5.43 ± 1.69; DPPH free antioxidant, 1.64 ± 0.94 mg/g and DPPH scavenging activity, 1.36 % [62].

Much later, the determination of the total phenolic content (TPC) of *E. indica* extracts using Folin-Ciocalteu reagent was also reported and the results showed that the methanol extract had the highest TPC (450 ± 210 GAE mg/g extract; $p < 0.05$) when compared with Dichloromethane (DCM), hexane and ethyl acetate (EA) extracts. On evaluation of the free radical scavenging ability of the extracts against DPPH free radical, the methanol extract showed the most free radical scavenging activity having 77.7% inhibition of the DPPH absorption. This is an excellent percentage of DPPH inhibition because BHT, being a synthetic free radical scavenger, did not exhibit 100% inhibition, possibly due to permanent residual absorption of 7% of the total absorption. When compared with methanol extract, ethyl acetate had 64.5 %, hexane 47.19% and dicloromethane 40.83 % free radical scavenging activities which implies that there is a strong and significant correlation between TPC and DPPH free radical scavenging activity of the *E. indica* extracts (Pearson correlation coefficient = 0.507; $p < 0.05$) [44].

Assay of the TPC of aqueous extract of *E. indica* and obtained 14.9 ± 0.002 mg/g total phenolic expressed as gallic acid equivalent per gram of extract (GAE; mg/g of extract). Also, the aqueous extract was able to reduce the stable DPPH in a dose-dependent manner and the half maximal effective concentration (IC50) was found to be 2350 µg/ml [63].

Using ethanol crude extract of aerial parts of *E. indica*, cellular antioxidant activities in whole blood, neutrophils and macrophages using chemiluminescence assay have been reported. The extract dose-dependently exhibited considerable inhibitory effect on the oxidative burst activities of the whole blood, neutrophils and macrophages. The ethanol extract produced – 17.90 – 36.90 % inhibition in whole blood, 0.00 – 46.70% in neutrophils when activated with zymosan-A, 35.60 – 74.50 % in neutrophils when activated with PMA and 39.60 – 63.40 % in macrophages [64].

Ethanol extract and n-hexane, chloroform, ethyl acetate, butanol and aqueous fractions of the whole plant of *E. indica* have been investigated for antioxidant activities. The effect of ethanol extract and its fractions on antioxidant enzymes was evaluated as well as estimation of lipid peroxidation product Malondialdehyde (MDA) and methaemoglobin generation of the extract and its fractions. The crude extract and its fractions exhibited significantly high levels of Superoxide dismutase in both serum sample and liver homogenate of albino rats

respectively, relative to control. A high level of reduced Glutathione enzyme was observed in animals pretreated with extract and its fractions in both serum and liver homogenate of albino rats when compared to control. Similarly, a high level of catalase enzyme was noted in both serum and liver homogenate of albino rats relative to control. Various degrees of antioxidant activity were observed with DPPH. The aqueous fraction showed the highest free radical scavenging activity depicted by percentage inhibition of DPPH absorption (79.0%) followed by n-hexane (77.3%), crude (69%), butanol (63.1%) and chloroform (51.3%). The fraction with the least free radical scavenging activity was ethyl acetate with 39.7% DPPH absorption. The level of malondialdehyde (MDA) in both serum sample and liver homogenate of the crude extract and its fractions respectively were high when compared to control. High levels of methaemoglobin generation were observed with the crude extract and its fractions when compared to control, the highest being n-hexane fraction. Lipid peroxidation, a degradative process of membrane lipids, measured indirectly by the degree of inhibition of malondialdehyde (MDA) by the plant extract and its fractions, was also slightly raised, that is, not reduced when compared to the control. MDA and methaemoglobin (MetHb) have oxidant potentials while SOD, CAT and GSH are antioxidant enzymes. The increase in levels of MDA and MetHb is evidence of the pro-oxidant effect of the extract and its fractions. Hence the extract and its fractions display a paradoxical role as pro-oxidants and antioxidants [58].

The DPPH radical scavenging activity, ABTS radical scavenging assay, NBT (superoxide radical scavenging) assay, reducing power assay, Cupric ions reducing antioxidant capacity (CUPRAC), Phosphomolybdate assay and antioxidant activity index of the aerial parts of *E. indica* ethanol and acetone extracts have recently been reported. The free radicals including DPPH, ABTS and SOR scavenging activity of the extracts was determined and compared with standard antioxidant (ascorbic acid). The lower the IC_{50}, the stronger the scavenging activity. In DPPH assay, IC50 value of ascorbic acid (positive control) was recorded as 16.913 ± 2.57 µg/ml. For DPPH radical scavenging activity, the IC_{50} values of free radical scavenging activities of E. *indica* for ethanol and acetone extracts were found to be 92.626 ± 8.13 µg/ml and 294.766 ± 2.70 µg/ml respectively. IC_{50} values of positive control (ascorbic acid) in ABTS assay was recorded as 2.804 ± 0.29 µg/ml. ABTS radical scavenging activity for ethanol and acetone extracts were 11.365 ± 3.70 µg/ml and 26.82 ± 5.58 µg/ml respectively. IC50 values of positive control (ascorbic acid) in SOR (Superoxide radical) assay was found to be 32.25 ± 4.67 µg/ml. SOR scavenging activity for ethanol and acetone extracts recorded 70.606 ± 9.28 µg/ml and 44.013 ± 5.45 µg/ml respectively. In reducing power assay and CUPRAC assay, the higher absorbance usually indicates stronger antioxidant activity. In determining the total phenolic contents, ethanol extract exhibited a higher and maximum contents (201.115 ± 11.61 mg GAE/g) while acetone exhibited a slightly little lower content. Similarly, ethanol extract exhibited a much higher flavonoids contents (32.621 ± 2.89 mg QE/g) than the acetone extract. Cupric ions reducing assay was expressed as gallic acid equivalent (mg GA/g extract) but cupric ion reducing power of the acetone plant extract was found to be higher (97.438 ± 1.38 mg/g) than that of ethanol extract. The results obtained from phosphomolybdate assay showed that ethanol extract had potent ability to reduce Mo (VI) to Mo (V) as compared to the acetone extract. Antioxidant activity index in terms of

percentage was determined for evaluation of antioxidant capacity and recorded ethanol extract approximately 46 % while acetone extract recorded approximately 42 % [65]. In DNA damage protection assay, the prevention of oxidative DNA damage by the plant extracts using p1391Z supercoiled DNA was also assessed. Control untreated DNA showed two bands, one of open circular DNA and one of supercoiled DNA. Combine treatment of DNA with Fenton reagent and quercetin (positive control) maintained the supercoiled DNA from scission while treatment of DNA with Fenton reagent without plant extracts led to the strand scission of the supercoiled DNA. DNA treated with different concentrations of plant extracts exhibited very little DNA damage protection activity. It was observed that the ethanol extract possessed an excellent DNA damage prevention activity while the acetone extract was not effective for DNA damage prevention. This is linked to greater flavonoid and phenol contents in the ethanol extract than the acetone extract. The DNA damage prevention was found to increase with increase in extract concentration [65].

Also, recently, hot water and ethanol extracts of *E. indica* leaves were screened for antioxidant activities using DPPH and the total phenolic contents using the Folin – Ciocalteu method determined. *E. indica* hot water extract exhibited higher DPPH radical scavenging activity of 69.62% while ethanol extract had 65.34%. The total phenolic content for hot water extract was 256.29 µg/g GAE while ethanol extract had 249.24 µg/g GAE [48].

Antileishmanial activity
The antileishmanial activity of ethanol crude extract against promastigotes of *Leishmania major* (DESTO) *in vitro* has been determined. Pentamidine and amphotericin B were used as standard antileishmanial compounds. The ethanol crude extract exhibited moderate antileishmanial activity against promastigotes of *Leishmania major in vitro* with ED_{50} of 58.18 ± 0.14 µg/ml while Pentamidine had 5.09 ± 0.04 µg/ml and Amphotericin B, 0.29 ± 0.05 µg/ml [37].

Also evaluated is the *in vitro* antileishmanial activity of methanol leaf extract of *E.indica* using Alamar blue assay. The assay was performed on a culture of Leishmania donovani promastigotes and axenic amastigotes. The growth of Leishmania promastigotes/amastigotes was determined. IC50 and IC90 values were computed from the dose–response curves. The result showed that the extract did not display any significant antileishmanial activity on the protozoan [54].

Cytotoxic activity
A research group screened 61 plant species widely used as anti-infective and anticancer agents in Malaysian indigenous medicine for antiviral and cytotoxic activities. One of these plants extracts was ethanol leaf extract of *E. indica*. The cytotoxicity assay was done using the HeLa (human cervical carcinoma) cell line. The assay used was the microtitration cytotoxicity assay. Various concentrations of the plant extracts were prepared from the stock solutions by serial dilution in RPMI-1640 medium to give a total volume of 100 µml in each well. Each well was filled with 100 µml of HeLa cell suspension in CGM at $1\text{-}2 \times 10^4$ cells/ml. Controls containing only HeLa cells were included for each sample. The assay for each concentration of plant extract was performed in triplicate and the culture plates were kept at 37°C with 5% (v/v) scope (low power), cytotoxicity was determined as the concentration of plant extract which reduced cell number by *ca.* 50% with reference to the control

(CD_{50}, mg/ml). CD_{50} is cytotoxic dose at 50%, i.e. the concentration of plant extract which reduced the number of HeLa cells by 50%. Cytotoxic activity was not present with the *E. indica* extract [66].

Other scientists used hexane, dichloromethane (DCM), ethyl acetate and methanol extracts of *E. indica* to evaluate their cytotoxic activity towards MCF-7 human breast cancer cells, HT-29 human colonic carcinoma cells and CEM-SS human cancer cell lines. The cytotoxicity assay results showed that the four extracts had IC_{50} values of >30 μg/ml 30 μg/ml and hence did not produce any cytotoxic effects on MCF-7, HT-29 and CEM-SS cancer cell lines after 72 h incubation when compared with the control RPMI 1640 and DMSO [44].

However, a different result was obtained when the crude ethanol extract of *E. indica* was evaluated for growth inhibitory and cytotoxic activity against HeLa cells (cervix cancer cell) by using the sulforhodamine-B assay. The result showed a weak cytostatic activity of the extract on HeLa cells. However the extract exhibited a moderate activity with an average percentage cytostatic activity of 54.03 ± 2.18% and average percentage inhibition of 45.77% but did not exert any cytocidal activity [64].

Methanol extract of *E. indica* has been used to determine cell viability with 3-(4, 5-dimethyl-2-thiazolyl)-2, 5 diphenyltetrazolium bromide (MTT) reagent. The absorbances were read on a multiwall spectrophotometer. The 50% cytotoxic concentration (CC_{50}) was defined as the sample concentration that reduced cell viability by 50% when compared to untreated controls. The MTT assay is often used to determine the cytotoxicity, proliferation and activation of potential medicinal agents based on the activity of mitochondrial dehydrogenase enzyme from living cell. An extract is considered nontoxic if the CC_{50} is higher than 0.02 mg/mL. The methanol extract of *E. indica* was observed to be toxic towards viability of Vero cells with IC50 of 3.2 mg/mL but still below safety concentration [67].

Using Brine shrimp lethality assay (BSLA), evaluation of the potential cytotoxic properties of the whole plant extracts of *Eleusine indica* has been investigated. The most common methods of extraction such as decoction, absolute ethanol and 50% water - 50% ethanol were tested to determine cytotoxic effects against the brine shrimp nauplii and the best extraction method was determined to be the use of 50:50 ethanol-water mixture extract. The acute lethal concentration (LC50) of the after 6 h exposure to mixture extract was 153.99 ppm, while the ethanolic extract obtained an LC50 of 409.73 ppm. Following 24 h exposure, increased mortalities of brine shrimps were observed in all prepared extracts. Maximum mortalities (100%) were recorded in the three concentrations of 100-1000 ppm in the mixture extract of *E. indica*. It was then concluded that *E. indica* possesses cytotoxic activity which suggests the presence of potential bioactive chemical compounds in the plant's extract [68].

A study determined the cytotoxic brine shrimp assay of *Eleucine indica* extracts using six different concentrations of the ethanol and acetone extracts (6, 12, 25, 50, 100 and 250 μg/mL). These were added in each vial and their final volume was made up to 5 mL using saline solution. After 24 h, ten shrimps were transferred to each vial and were incubated at 32 °C for 24 h, after which the survivors were counted. LC50 values and % mortality were calculated. Vincristine sulfate was used as a positive control and its LC50 value was recorded as 0.839 ppm. *Eleucine indica* ethanol and acetone extracts revealed moderate cytotoxic activity with ethanol extract having lethal concentration (LC$_{50}$) of 16.857 ppm

whereas acetone extract had LC$_{50}$ of 9.828 ppm. The degree of lethality was directly related to the concentration of extract. The observed lethality of the plant extracts to brine shrimps depicted the presence of cytotoxic compounds in this plant. It has been reported that plant extracts with LC50 values below 20 μg/ml have more probability of producing anticancer compounds [65, 69].

Flavonoid and phenolic bioactive constituents have been known to exert inhibitory effects against multiple viruses and bacteria and possess free radical scavenging and anticancer activity. Also, saponins do possess potent antimicrobial, antioxidant and cytotoxic potential in plants [70, 71].

Screening of methanol leaf extract of *E. indica* for cytotoxicity against transformed human monocytic (THP1) cells has been studied which the standard fluorescence was measured on a fluorometer at 544 nm excitation and 590 nm emission. The result showed that the extract did not display cytotoxicity on transformed human monocytic (THP1) cells [54].

Antitrypanosomal Assay
Recently, antitrypanosomal assay of the methanol leaf extract of *E.indica* was asssessed. Two-day old culture of Trypanosoma brucei, in the exponential growth stage, was diluted with Iscove's Modified Dulbecco's medium (IMDM) to obtain 5000 parasites/mL. Ninety-six well microplates were used to conduct the assay. For primary screening, extract stock solution of 20 mg/Ml was used and dilutions (1 mg/mL) were made in IMDM. Each well got 4 μL of diluted extract and 196 μL of the culture medium to have a final volume of 200 μL. Thereafter, plates were incubated at 37 °C in 5% CO2 for 48 h. After, ten microliter (10 μL) of alamar blue was added into each well and plates and left to incubate overnight. Standard fluorescence was measured on a Fluostar Galaxy fluorometer at 544 nm excitation and 590 nm emission. The standard drug used as control was Pentamidine. Furthermore, secondary screening to evaluate dose–response analysis was done using concentrations ranging from 10 to 0.4 μg/mL and IC50 and IC90 values were computed from dose response growth inhibition curve by XLfit version 5.2.2. The result shows that methanol leaf extract of E. indica had 97 % inhibition against Trypanosoma brucei at a concentration of 20 μg/mL and was active against T. brucei brucei with IC50 and IC90 of 8.26 and 10.14 μg/mL, respectively [54].

Antiviral Assay
A study was conducted on the antiviral activity of ethanol leaf extract of *E. indica* against Herpes simplex virus type-1 (HSV-1) and vesicular stomatitis virus (VSV), which are DNA and RNA virus type respectively. The simplified plaque reduction assay was used. Microtitre plates with confluent monolayer cultures of Vero cells were inverted to remove spent medium. Each well was filled in triplicate with 100 μl of the extract serially diluted in RPMI-1640 medium. Then was added 100 μl of medium containing *ca.* 30 plaque forming units (pfu) of HSV-1 or 10 pfu of VSV, per well of confluent Vero cells. In each plate, wells used for controls consisted of two treatments namely: those cells not treated with extract and virus and those treated only with virus. Incubation of plates followed for 66 h (HSV-1) and 36 h (VSV) at 37°C. The low power of an inverted microscope was used to score antiviral activity as the non-cytotoxic minimum inhibitory concentration (MIC, mg/ml) which totally prevented cytopathic effects. The result showed selective antiviral activity against only HSV-1 with MIC of 0.1 mg/ml.

This selective antiviral activity of the extract against either HSV-1 or VSV depicts the involvement of different mechanisms of action due to the difference in nucleic acid composition of the viruses [66].

Also, another study further screened the methanol and n-hexane whole plant extracts for antiviral activity against HSV-1 clinical strain and Vero cells. Vero cells were maintained in Dulbecco's Modified Essential Medium (DMEM) supplemented with 5% Fetal Bovine Serum penicillin/streptomycin (100 U/L), MEM non-essential amino acid and amphostate B (20 U/L) and the cell culture being maintained in an incubator at 37°C and humidified in 5% CO_2 atmosphere. HSV-1 was propagated in Vero cells and incubated until cytopathic effects developed. Percentage plaque inhibition was calculated. The EC50 was calculated as the concentration of extracts that inhibits 50% of virus plaque relative to control. Selective index (SI) was calculated as the ratio of the CC50 to EC50 values (CC50/EC50) to depict the safety of drug to be used as medicine. The result obtained shows that the selective indices (SI = CC50 / EC50) for both methanol extract and hexane fractions were 12.2 and 6.2 respectively, which demonstrate that the plant has antiviral property [67].

Antimicrobial Activities
The antimicrobial activity of *E. indica* extracts using two Gram-positive bacteria, methicillin resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* B29, and other two Gram-negative bacteria, *Pseudomonas aeruginosa* 60690 and *Salmonella choleraesuis* has been studied. The extracts used were hexane, dicloromethane (DCM), ethyl acetate (EA) and methanol. Streptomycin (10 μg/disc) was used as the standard. Screening of antibacterial activity was carried out by determining the zone of inhibition using the Paper Disc Diffusion method. The highest antibacterial activity observed was obtained by the hexane extract on MRSA, while the DCM extract showed weak activity on *P. aeruginosa*. On the other hand, the EA extract showed a broad spectrum activity, compared with the positive control (Streptomycin) against all tested bacteria except for *B. subtilis*, which showed resistance to all extracts of EI. In contrast, methanol extract did not exhibit any antibacterial activity towards MRSA, *B. subtilis* B29, *P. aeruginosa* 60690 and *S. choleraesuis* [44].

Further evaluation of the antimicrobial activities of *E. indica* used methanol, ethanol, hexane, ethyl acetate, aqueous, mixture methanol/water and ethanol/water extracts. Disc diffusion assay method was used for the Antibacterial and antifungal assay. The clinical microorganisms included were the most common strains implicated in gastro-intestinal disorder such as diarrhea and dysentery. Because the plant is used traditionally to treat gastrointestinal disorder, the choice of the microorganism was oriented by this fact. They were 15 bacterial and 2 yeast stains: *Escherichia coli (E. coli), Klebsiella pneumoniae, Shigella dysenteriae type 1, Shigella flexneri, Morganella morganii, Proteus vulgaris, Proteus mirabilis, Salmonella typhi, Citrobacter freundii, Enterobacter cloacae, Enterobacter agglomerans, Staphylococcus aureus, Streptococcus feacalis, Pseudomonas aeruginosa, Bacillus cereus T, Candida albicans* and *Candida glabrata*. MIC and MBC values were also determined. The result shows that methanol extract has stronger and broader spectrum of antimicrobial activity as compared to hexane, water or ethanol extract. The methanol extract showed moderate activity against *Salmonella typhi, Staphylococcus aureus* and *Streptococcus faecalis*. The methanol/water

mixture showed moderate activity against *Staphylococcus aureus*. The ethanol extract also showed moderate activity against *Bacillus cereus, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The ethanol/water extract only showed activity against *Proteus vulgaris*. The ethyl acetate extract had moderate activity against *Staphylococcus aureus* and *Bacillus cereus* while the water extract had no activity. The Ethyl acetate extract gave MIC of 78 μg/ml and MBC of 400 μg/ml, for staph aureus. It was considered that if the extracts displayed an MIC less than 100 μg/ml, the antimicrobial activity was good. It was also considered that if the extracts displayed an MIC of 8 mg/ml or below against any of the yeast tested, the antimicrobial activity was good. But none of the extracts showed activity against fungal organisms [36].

Another evaluation of the antimicrobial activity of methanol and chloroform extracts of *E. indica* used the agar diffusion method. The bacterial and fungal organisms used were *S. aureus*, *E. coli, Enterobacter aerogens, P. Vulgaris, Streptococcus specie, Bacillus specie, Pseudomonas aerogenes, Klebsiella aerogenes, Aspergillus flavus, Aspergillus niger* and *Candida albicans*. The minimum inhibitory concentration of the extract against these microorganisms was carried out using glucose indicator broth. To determine the minimum inhibition concentration and minimum fungicidal concentrations of the extracts, punched agar diffusion method was applied. The result of the antibacterial test showed that both were active against all the test organisms with the highest activity shown with *Enterobacter aerogenes* having 38mm and 33mm average diameter zones of inhibition. When tested against fungal organisms, both chloroform and methanol extracts were observed to effectively inhibit the growth of all three fungi with the highest inhibition seen with *candida albican* giving average diameter of zone of inhibition 20mm for chloroform and 18mm for methanol extracts respectively. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the two extracts confirmed that they were active at very low concentrations. The compounds isolated from the chloroform and methanol extracts were found to be derivatives of Hexadecanoic acid, phosphatidylethanolamine and ethanediyl ester. Thy are 1 – [[[(2- aminoethoxy)hydroxyphosphinyl]oxy]methyl]1-,2-ethandiylester for chloroform and Hexadecanoic acid for methanol extracts respectively. Both compounds are known to have anticancer, antifungal and anticonvulsant and antiviral properties [10].

Further screening of antibacterial activity was done with methanol, ethanol and aqueous extracts of the aerial part of *E. indica* against *Shigella dysenteriae, Escherichia coli, Samonella typhi, Staphylococcus aureus, Streptococcus facecalis* and *Lactobacillus lactis*. The antimicrobial sensitivity was by the disc diffusion method. The aqueous extract was the most susceptible while the ethanol extract was the least susceptible to these microorganisms. The observed antimicrobial activity was concentration dependent [46].

In vitro antimicrobial activity of whole-plant extracts of *Eleucine indica* has been investigated. Four typed cultures of bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and one clinical strain of fungi, *Candida albicans*, were assessed using agar well diffusion method. Drugs used as in the test as positive controls were streptomycin 0.4mg/ml and Nystatin 50,000 I.U/ml. The extracts that exhibited antimicrobial activity were then tested to determine the Minimum Inhibitory

Concentration (MIC) for each bacterial or fungal sample. The ethyl acetate extract showed the widest zone of inhibition (25.0 mm), followed by chloroform when tested against *Staphilococcus aureus.* The ethyl acetate extract also exhibited broad spectrum antibacterial activity against *Pseudomonas aeruginosa*, *E. coli* and *B. subtilis.* None of the extracts showed any inhibitory effect against the fungal strain of *Candida albicans.* Both chloroform and ethyl acetate extracts exhibited similar potencies having MIC of 50 mg/ml against *E. coli* and *P. aeruginosa* respectively [72].

Another study screened the microbial growth inhibition potential of *E. indica* methanol leaf and root extracts using the agar disc diffusion method. The MIC of the methanol crude extracts against clinical isolates of bacteria associated with skin and oral infection was determined. Standard strains used in the study included *S. aureus* and *E. coli*, and clinically isolated strains of *K. pneumoniae* and *P. mirabilis.* The result showed that the *E. indica* extracts were more active against *S. aureus* and *P. mirabilis* than with other organisms. Minimum inhibitory concentration (MIC) of the extracts against bacterial strains associated with oral and skin infections were 256 μg/mL (*S. aureus*), 512 μg/mL (*K. pneumoniae*), 256 μg/mL (*P. mirabilis*) and 512 μg/mL (*E. coli.*) respectively. The observed antibacterial activity may be attributed to a wide variety of secondary metabolites especially phenolics and flavonoids that have been reported with potent antimicrobial properties [73].

Further report on the antimicrobial activities of *Eleusine indica* used ethanol and acetone extracts of. Bacterial strains used include *S. aureus, L. monocytogenes, B. spizizenii, S. typhi* and *E. coli* while fungal pathogens include *W. anomalus, F. oxysporum, Mucor sp., A. flavus, A. niger* and *S. cerevisae.* Bacterial isolates were sub-cultured in a nutrient broth and then incubated at 37 °C for 18 h while the fungal isolates were sub cultured on SDA media for 24 h at 25 °C. Antibacterial and antifungal assay were performed via agar disc diffusion method. Oxytetracycline and Chloramphenicol were used as positive control. Results showed that the plant extracts were active against *L. monocytogenes, W. anomalous, A. flavus* and Mucor specie while *S. aureus, F. oxysporum* and *A. niger* were revealed as highly resistant pathogens. The ethanol extract recorded low MIC of 50 μg/ml against *B. spizizenii* among bacterial strains [65].

Recent evaluation of this plant tested the methanol leaf extract of *E. indica* against fungal and bacterial organisms. Fungal organisms include *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus* while bacterial organisms were *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and Vancomycin resistant *Enterococci faecalis. The in vitro* antimicrobial assay was done using a modified version of the CLSI methods. Drug controls were included in the secondary assay; ciprofloxacin for bacteria and amphotericin B for fungi. Primary *in vitro* antimicrobial activity of methanol extracts of E. indica showed that the primary assay with P. falciparum D6Pinh gave percentage inhibition of 37 %. The extract revealed antifungal activity with *Candida albicans* (0 %), *Aspergillus fumigatus* (1 %) and *Cryptococcus neoformans* (5 %) while antibacterial activity revealed MSRA (0 %), *Escherichia coli* (7 %) *Pseudomonas aeruginosa,* (1 %) *Klebsiella pneumoniae* (0 %) and Vancomycin resistant *Enterococci faecalis* (5 %) respectively [54].

Antihypertensive property
In vivo anti-hypertensive activity of petroleum ether, chloroform, methanol and ethanol extracts of *E. indica* has been reported using adrenaline to induce hypertension in rats by non-invasive tail-cuff method. Hypertension was induced in rats after being anaesthetized with diethyl ether and 0.1 ml of adrenaline was injected into rats by intra-peritoneal injection for 10 consecutive days. To confirm the induction of hypertension, different hemodynamic parameters were measured by using Non-invasive tail cuff method with CODA Non-Invasive Blood Pressure System. Then blood pressure was measured by Non-invasive Tail cuff. The rat was kept in strainer and the tail cuff was applied on the tail of rat for determination of blood pressure. Normal blood pressures of all rats were recorded as baseline blood pressure. After that the animals were treated with respective treatment and again blood pressure was recorded as after drug treatment. The systolic blood pressure and diastolic blood pressure displayed on monitor were recorded. In order to evaluate anti-hypertensive effect of drugs, adrenaline was injected after 5 minutes. Again the blood pressure was recorded and the difference between baseline blood pressure and blood pressure after adrenaline treatment were calculated and compared. The results revealed that the extracts had antihypertensive activity with the ethanol extract having the most significant antihypertensive activity and chloroform showing weak antihypertensive effect [52].

Anthelminthic Property
Ethanol, methanol and aqueous extracts of *E. indica* have been assessed for anthelminthic activity. The *in vitro* method used was a modified coproculture using the filter paper culture technique. Children's stool samples were collected. Stool sample (1.0g) was smeared on a slide, diluted, covered with a cover slip and observed under the microscope at x40 and x100 magnification. Stool sample I from a 1 ½ year old girl contained 4 living *Strongyloides stercoralis* and many larvae. The second stool from a 2 ½ year old boy contained only the larval stages of the worm. Each stool (0.1g) was smeared on different filter papers. The different filter papers were dipped into separate solutions of the three plant extracts at concentrations of 0.15, 0.3, 0.6, 1.2, 2.4 and 4.8mgcm-3 and kept for 4 days. They were then air dried for 55h, after 4 days, and viewed under the microscope. The result showed that the plant had marked anthelmintic activity against *Strongyloides stercoralis*. At the levels of 2.4 and 4.8mgcm-3 of the three extracts, all the worms and their larvae were completely dead. It was observed that survival of the worm was concentration-dependent: the higher the concentration of the extract, the lower the proportion of the worm that survived [46].

Anticonvulsant Property
Investigation into the anticonvulsant potentials of ethanolic extract of *Eleusine indica* has also been reported. Albino Wistar mice were separated into five groups with six animals in each group and then pretreated with distilled water, various doses of the extract (200–600 mg/kg) and standard drug diazepam (0.5 mg/kg). After thirty minutes, pentylenetetrazole (70 mg/kg), aminophylline (280 mg/kg) and isoniazid (250 mg/kg) were used to induce convulsions by intraperitoneal administration. These mice were then placed in plexiglas cages and monitored for the occurrence of

seizures over a thirty-minute time period. The latency of convulsions, duration of tonic convulsions and mortality protection were recorded. The results showed that the extract exhibited a dose-dependent increase in the latency of clonic convulsions and decrease in duration of tonic convulsions as compared to the control and these effects were statistically significant $p < 0.001$). The extract also provided protection against the mortality which was similar to that produced by the standard drug diazepam. The significant increase in the latency of clonic convulsions and decrease in duration of tonic convulsions caused by the extract depicted anticonvulsant activity [74].

Antiurolithiatic activity
A study examined the antiurolithiatic potential of *Eleusine indica* ethanolic extract in rats using. Ethylene glycol (0.75% w/v) for inducing urolithiasis, and Cystone (750mg/kg) as the standard herbal supplement. The effects of *E. indica* ethanolic extract (200 and 400mg/kg) were compared with Cystone as the standard and distilled water as the negative control. *Eleusine indica* crude extract was found to be inactive as an antiurolithiatic agent despite some significant values based on the parameters measured. No significant decrease in the calculogenic substances to treat the renal impairment formed was observed. Altthough some of the parameters measured, such as urinary pH, urine volume at 200mg/kg and serum BUN, were found to be significant, those results alone are insufficient to support its antiurolithiatic activity [75].

Subchronic Toxicity studies
The subchronic toxicological effects of the extract in adult albino Wistar rats have been evaluated. Rats of both sexes were randomized into 5 groups of 6 animals per group and orally administered with extract (200, 400 and 600 mg/kg) for groups 2–4, respectively. Group 1 received distilled water (10 mL/kg) orally and served as negative control while group 5 was administered with 100 mg/kg of silymarin orally. Drugs were administered on alternate days for 28 days at 09.00 am. Toxic manifestations and mortality were monitored daily and weight changes of animals were recorded every week. On day 29, after an overnight fast, the animals were weighed, anaesthetized with light chloroform. Blood samples were collected by cardiac puncture for haematological and biochemical analyses. Autopsy was performed noting any macroscopic abnormalities and the brain, heart, liver, spleen, kidney and lungs were weighed immediately after removal and fixed in 10% formalin and kept in that solution for further histopathological examination. The results showed that haematological indices were preserved but the extract showed significant haemostatic potentials. There was significant reduction in total bilirubin, aspartate aminotransferase, alanine transaminase, alkaline phosphatase and blood glucose compared to control. The level of total protein was found to increase significantly. Kidney functions were unaffected. This suggests that prolonged consumption of this extract reduces both bleeding and clotting times and reduces blood sugar Furthermore, organ weights were not affected but animal weights increased significantly. Relative organ weights were not affected. The extract caused, at low doses, slight inflammation of the liver, spleen, lungs, kidneys and brain. With high dose of the extract, the spleen and lungs showed moderate inflammation. The lungs also showed moderate interstitial fibrosis [76, 77].

Table 1: Phytochemical Screening of *Eleusine indica* leaf extracts

Phytochemicals	Methanol	n-Hexane	Aqueous	Acetone	Ethanol	Ethyl Acetate
Flavonoids	+++	-	++	++	++	++
Tannins	+	+	-	++	-	-
Saponins	-	-	-	-	-	+
Alkaloids	++	+	+++	++	++	-
Cardiac glycosides	++	-	+	-	+	-
Anthracene glycosides	++	-	-	-	+	-
Anthraquinones	+	+	+	-	++	-

Key: +++ = high concentration; ++ = medium concentration
+ = low concentration; - = absence

Conclusion
Eleusine indica (L.) Gaertn. has been of great use in folk medicine in the treatment of various disease conditions and some of the studies done on this plant, spanning many years now, have shown this plant to be a medicinal laboratory with pharmacotherapeutic potentials. Further studies on the compounds so far isolated need to be done to establish their structure-activity relationships and elucidate mechanisms of action. Clinical trials would unveil the therapeutic benefits of this plant and make for its full exploitation.

Acknowledgements

References
1. Moyo M, Aremu AO, Van Staden J. Medicinal plants: an invaluable, dwindling resource in sub-Saharan Africa. J Ethnopharmacol. 2015a; 174:595-606. http://dx.doi. org/10.1016/j.jep.2015.04.034.

2. WHO. WHO Traditional Medicine Strategy. World Health Organization, Geneva, Switzerland (Online publication), 2014-2023. 2013 ⟨http://apps.who.int/iris/bitstream/10665/92455/1/978924 1506090_eng.pdf⟩

3. WHO. WHO World Health Statistics 2010. World Health Organization, Geneva, Switzerland (Online publication), 2010. ⟨http://www.who.int/gho/publications/world_health_stati stics/EN_WHS10_Full.pdf⟩.

4. Moyo M, Aremu AO, Van Staden J. Ethnopharmacology in sub-Saharan Africa: current trends and future perspectives. In: Heinrich, M., Jäger, A.K. (Eds.), Ethnopharmacol. John Wiley & Sons Ltd, Chichester, UK, 2015b.

5. Vlietinck AJ, Pieters L, Apers S, Cimanga K, Mesia K, Tona L. The value of central-African traditional medicine for lead finding: some case studies. J Ethnopharmacol. 2015; 174:607-617.

6. Bisht MS, Muka Y. Genome organization and polyploid evolution in the genus Eleusine (Poaceae). Plant Sys

Evol. 2002; 233:243-258.

7. Fage JD, Oliver RA. Paper in African Prehistory. Cambridge University Press. Cambridge, 1960, 130.

8. Cope TA. Poaceae. In: Nasir E, Ali SI, editors. Flora of Pakistan. 1982; 143:26-27.

9. Haber RM, Seaman MT. Two new records from Lebanon: Chamacyse nutans (Lag.) Small (Euphorbiaceae) and Eleucine indica (L) Gaertus (Poaceae). Turkish J Bot. 2007; 31:341-343.

10. Alaekwe IO, Ajiwe VIE, Ajiwe AC, Aningo GN. Phytochemical and Anti – Microbial Screening of the Aerial Parts of *Eleusine indica.* Int'l J Pure and Appl. Biosci. 2015; 3(1):257-264.

11. Ettebong EO, Nwafor PA, Okokon JE. *In vivo* antiplasmodial activities of ethanolic exract and fractions of Eleucine indica. Asian Pacific J Trop. Med. 2012, 673-676.

12. Pattanayak S, Maity D. Use of *Eleusine indica* (L.) Gaertn. (Kechila Ghas) as an antipyretic medicine of herbivores. Explor Anim Med Res. 2017; 7(1):94-96.

13. Burkill H.M. The Useful Plants of Tropical West Africa, Royal Botanical Gardens, Kew, London, 1995; 1:293-295.

14. Salimanth SS, de Oliviera AC, Goden IP, Bennetzen JL. Assessment of Genome Origins and Genetic Diversity in the Genus Eleusine with DNA Markers. Genome. 1995; 38(4):757-763.

15. Al-Zubairi AS, Abdul AB, Abdelwahab SI, Peng CY, Mohan S, Elhassan MM. *Eleusine indica* Possesses Antioxidant, Antibacterial, and Cytotoxic Properties. Evidence-based Compl. Alternat Med, 2011. doi: 10.1093/ ecam/nep091. Retrieved on 26 October 2016 from NCBI website: https://www. ncbi.nlm.nih.gov/pmc/articles/PMC3137868/

16. Ettebong EO. Antiplasmodial Activities of Ethanolic Extract of *Eleucine indica* L. Gaertn (Poaceae) – Ph.D Thesis, 2014, 23-24.

17. Waterhouse DF. The major arthropod pests and weeds of agriculture in Southeast Asia. ACIAR Monograph No. 21. Canberra, Australia: Australian Centre for International Agricultural Research, 1993, 141.

18. Standifer LF, Wilson PW, Porche-Sorbet R. Effects of solarization on soil weed seed populations. Weed Sci. 1984; 32(55):69-73. ISSN 0043-1745.

19. Rojas-Sandoval J, Acevedo-Rodríguez P. Invasive Species Compendium, Eleusine indica (goose grass), Department of Botany-Smithsonian NMNH, Washington DC, USA, 2014.

20. Queensland Department of Primary Industries and Fisheries. Special edition of Environmental Weeds of Australia for Biosecurity Queensland, Australia: The University of Queensland and Department of Primary Industries and Fisheries, 2011. http://keyserver.lucidcentral.org/weeds/data/03030800-0b07-490a-8d04-0605030c0f01/media/Html/Index.htm. Date of access 3rd May, 2020.

21. Jingchao C, Hongjuan H, Shouhui W, Zhaofeng H, Xu W, Chaoxian Z. Investigating the mechanisms of glyphosate resistance in goosegrass (*Eleusine indica* (L.) Gaertn.) By RNA sequencing technology. The Plant J. 2017; 89:407-415.

22. Nishimoto RK, Mccarty LB. Fluctuating temperature and light influence seed germination of goosegrass (*Eleusine indica*). Weed Sci. 1997; 45:426-429.

23. Holm LG, Plucknett DL, Pancho JV, Herberger JP. The World's Worst Weeds. University Press, Honolulu, HI, USA, 1997, 609.

24. Lourens J, Arceo M, Datud F. Fenoxaprop-ethyl (Whip) and fenoxaprop-P-ethyl (Whip S) for grass control in direct seeded rice under rainfed upland conditions in the Philippines. In: Proceedings, 12th Asian-Pacific Weed Science Society Conference. Asian-Pacific Weed Science Society, 21–26 August. The Organization of the 12th Asian-Pacific Weed Science Society, Seoul, Korea, 1989, 291-301.

25. Valverde BE, Chaves L, Gonzales J, Garita I. Field evolved imazapyr resistance in Ixophorus unisetus and Eleusine indica in Costa Rica. Brighton crop protection conference, weeds. Proceedings of an international conference, Brighton, UK, British Crop Protection Council (BCPC). 1993; 3:1189-1194.

26. Baird WMV, Morejohn L, Zeng L, Mysore K, Kim H. In: Brown H., Cussans G.W., Devine M.D., Duke S.O., Fernandez-Quintanilla C., Helweg A., Labrada R.E., Landes M., Kudsk P., Streibig J.C., Genetic, molecular and biochemical characterization of dinitroaniline herbicide resistance in goosegrass (Eleusine indica). Proceedings of the Second International Weed Control Congress, Copenhagen, Denmark. 1996; 1-4:551-557.

27. Doll J. Glyphosate resistance in another plant. Resistant Pest Management. 2000; 11(1):5-6.

28. Jalaludin A, Ngim J, Bakar BHJ, Zazali Alias. Preliminary findings of potentially resistant goosegrass (*Eleusine indica*) to glufosinateammonium in Malaysia. Weed Biology and Management. 2010; 10(4):256-260.

29. Mueller TC, Barnett KA, Brosnan JT, Steckel LE. Glyphosate-resistant goosegrass (*Eleusine indica*) confirmed in Tennessee. Weed Science. 2011; 59(4):562-566.

30. Vargas L, Ulguim AR, Agostinetto D, Magro TD, Thürmer L. Low level resistance of goosegrass (*Eleusine indica*) to glyphosate in Rio Grande do Sul-Brazil. Planta Daninha. 2013; 31(3):677-686.

31. An, Jing, Shen XueFeng, Ma QiBin, Yang CunYi, Liu SiMin, Chen Yong. Transcriptome profiling to discover putative genes associated with paraquat resistance in goosegrass (*Eleusine indica* L.). PLoS ONE. 2014; 9(6):099940.

32. Penescu A, Bran M, Nichita M, Ionescu N, Săndoiu DI, Ciontu C *et al*. Comparative study regarding the influence of herbicides on the yield of sunflower crops, the crops being obtained with conventional, clearfield and expres sun technologies in the field conditions of Moara Domneasca. Scientific Papers. Series A. Agronomy. 2018; LXI(1). ISSN 2285-5785, ISSN CD-ROM 2285-5793, ISSN ONLINE 2285-5807, ISSN-L 2285-5785.

33. Gruyal GA, del Roasario R, Palmes ND. Ethnomedicinal plants used by residents in Northern Surigao del Sur, Philipines. Nat Prod Chem Res. 2014; 2:140. doi:10.4172/2329-6836.1000140

34. Tutor JT, Chichioco-Hernandez CL. Angiotensin-Converting Enzyme Inhibition of Fractions from *Eleusine indica* Leaf Extracts. Pharmacog J. 2018; 10(1):25-8.

35. Teodora DB, Kryssa DB. Ethnomedicinal plants in Bayabas, Sablan, Benguet Province, Luzon, Philipines. Electronic J Biol. 2015; 11(3):63-73.

36. Mabeku LBK, Roger KJ, Louis OEJ. Screening of Some Plants Used in the Cameroonian Folk Medicine for the Treatment of Infectious Diseases. Int'l J Biol. 2011;

3(4):13-21.

37. Okokon JE, Udoh AE, Etebong EO, Azare BA. Antioxidative burst, cytotoxic and antileishmanial activities of *Eleusine indica*; Int'l J Pharmacol Res. 2014; 4(3):165-168.

38. Kakoli RB, Tania K, Mirza NM, Auditi S, Tasneema I, Mehreen R *et al*. Medicinal plants used by folk medical practitioners of four adjoining villages of Narail and Jessore districts, Bangladesh. American-Eurasian J. Sust. Agric. 2011; 5(1):23-33.

39. De Melo GO, Muzitano MF, Legora-Machado A, Almeida TA, De Oliveira DB, Kaiser CR *et al*. Cglycosylflavones from the aerial parts of Eleusine indica inhibit LPS-induced mouse lung inflammation. Planta Med. 2005; 71(4):362-363.

40. Sagnia B, Fedeli D, Casetti R, Montesano C, Falcioni G, Colizzi V. Antioxidant and antiinflammatory activities of extracts from Cassia alata, *Eleusine indica*, Eremomastax speciosa, *Carica papaya* and *Polyscias fulva* medicinal plants collected in Cameroon. PLoS One. 2014; 9(8):1-10.

41. Upasani MS, Upasani SV, Beldar VG, Beldar GC, Gujarathi PP. Infrequent use of medicinal plants from India in snakebite treatment. Integr. Med. Res, 2018; 7:9-26.

42. Chowdhury T, De Sarker D, Roy SC. Local folk use of plants in Dakshin Dinajpur district of West Bengal, India. Intern Res J Biol Sci. 2014; 3(5):67-79.

43. Obico JJA, Ragragio EM. A survey of plants used as repellents against hematophagous insects by Ayta people of Porac, Pampanga province, Philipines. Philippine Sci Lett. 2014; 7(1):179-186.

44. Adel SA, Ahmad BA, Siddig IA, Chew YP, Syam M, Manal ME. *Eleucine indica* possesses Antioxidant, Antibacterial and Cytotoxic properties. Hindawi Publishing Corporation. Evidence-Based Compl Alternat Med, 2011. Article ID 965370, doi:10.1093/ecam/nep091:1-6.

45. Regmi PR, Devkota NR, Timsina J. Re-growth and nutritional potentials of *Eleusine indica* (L) Gaertn. (Goose grass). J Inst Agri Anim Sci. 2004; 25:55-63.

46. Morah FNI, Otuk ME. Antimicrobial and anthelminthic activity of *Eleusine indica*. Acta Sci Intellect. 2015; 1(4):28-32.

47. Babu RH, Savithramma N. Studies on mineral analysis of grasses of Poaceae. Intern J Pharma Sci. 2014; 4(3):526-531.

48. Valentino MJG, Dulay RMR, Lindain JMG. Phytochemicals, anti-oxidant potential and total phenolics of *Eleusine indica* leaf extracts. International Journal of Biosciences. 2018; 13(5):317-321.

49. Iberahim R, Wan Ahmad Yaacob, Ibrahim N. Phytochemistry, cytotoxicity and antiviral activity of Eleusine indica (sambau), AIP. Conference Proceedings. 2015; 1678:030013.
Doi: http://dx.doi.org/10.1063/1.4931234.

50. Agrawal PK. Carbon-13 NMR of Flavonoids. Amsterdam, The Netherlands: Elsevier Science Publishers B.V, 1989, 332-333.

51. Phuong NM, Sung TV, Ripperger H, Adam G. Sterol Glucosides from *Eleusine indica*. Planta Med. 1994; 60:498.

52. Desai V, Patil VM, Patil SS, Kangralkar VA. Phytochemical Investigation of *Eleusine indica* for In-Vivo Anti-Hypertensive Activity. International Journal of Innovative Science and Research Technology. 2017; 2(6):405-416.

53. Okokon JE, Okokon PJ, Sahal D. *In vitro* antiplasmodial activity of some medicinal plants from Nigeria. Int'l J Herb Med. 2017; 5(5):102-109.

54. Ogbole OO, Segun PA, Fasinu PS. Antimicrobial and antiprotozoal activities of twenty-four Nigerian medicinal plant extracts. South African J Bot. 2018; 117:240-246.

55. Builders M, Tarfa F, Aguiyi J. The Potency of African Locust Bean Tree as Antimalarial. J Pharmacol Toxicol. 2012; 7:274-287.

56. Ettebong EO, Nwafor PA. Anti-inflammatory and analgesic potentials of *Eleucine indica*. The J Phytopharmacol. 2014; 3(2):130-138.

57. Obute GC. Ethnomedicinal plant resources ofsouth eastern Nigeria. African J Interdiscipl Studies. 2007; 3(1):90-94.

58. Ettebong EO, Nwafor PA. Antipyretic and antioxidant activities of *Eleucine indica*. The J Phytopharmacol. 2015; 4(4):235-242.

59. Sultana S, Asif HM, Akhtar N, Ahmad K. Medicinal plants with potential antipyretic activity: A review. Asian Pacific J Trop Dis. 2015; 5(1):202-208.

60. Pal RS, Ariharasivakumar G, Girhepunjhe K, Upadhay A. *In-vitro* antioxidative activity of phenolic and flavonoids compounds extracted from seeds of Abrus precatorius. International J. Pharm Pharmaceut Sci. 2009; 1:136-140.

61. Bhattacharjee S, Waqar A, Barua K, Das A, Bhowmik S, Debi SR. Phytochemical and pharmacological evaluation of methanolic extract of *Lathyrus sativus* L. seeds. Clin Phytosci. 2018; 4:20.

62. Agbor GA, Kuate D, Oben JE. Medicinal plants can be good source of antioxidants: Case study in Cameroon. Pakistan J Biol Sci. 2007; 10(4):537-544.

63. Iqbal M, Gnanaraj C. *Eleusine indica* L possesses antioxidant activity and precludes carbon tetrachloride (CCl4)-mediated oxidative hepatic damage in rats. Environ Health Prev Med. 2012; 17:307-15.

64. Okokon JE, Udoh AE, Ettebong EO, Azare BA. Antioxidative Burst, Cytotoxic and Antileishmanial Activities of *Eleuscine indica*. Int'l J Pharmacol Res. 2014; 4(3):165-168.

65. Iram F, Sobia K, Tariq M. Evaluation of biological potential of selected species of family Poaceae from Bahawalpur, Pakistan. BMC Compl Alternat Med. 2018; 18:27. DOI 10.1186/s12906-018-2092-1

66. Abdul MA, Mackeen MM, Elsharkawyl SH, Hamidi JA, Ismaili NH, Ahmadi FBH *et al*. Antiviral and cytotoxic activities of some. Plants used in Malaysian indigenous medicine. Pertanika J Trop. Agric. Sci. 1996; 19(2/3):129-136.

67. Rashidah I, Wan AY, Nazlina I. Phytochemistry, Cytotoxicity and Antiviral Activity of *Eleusine indica* (Sambau). The 2015 UKM FST Postgraduate Colloquium. AIP Conf. Proc. 2015; 1678:030013-1-030013-4; doi: 10.1063/1.4931234

68. Responte MA, Maria R, Dacar B, Olga MN, Mylene MU. Brine shrimp lethality assay of whole plant extracts of *Eleusine indica*. Adv Agric Botanics Int J Bioflux Soc. 2015; 7(2):90-5.

69. Wong FC, Tsun-Thai C, Yee-Wei H. Antioxidation and cytotoxic activities of selected medicinal herbs used in Malaysia. J Med Plants Res. 2012; 6(16):3169-75.

70. Montoro P, Braca A, Pizza C, De Tommasi N. Structure-

antioxidant activity relationships of flavonoids isolated from different plant species. Food Chem. 2005; 92:349-55.

71. Negi JS, Negi PS, Pant GJ, Rawat MSM, Negi SK. Naturally occurring saponins: chemistry and biology. J Pois Med Plant Res. 2013; 1(1):001-6.

72. Ettebong EO, Bassey AI. *In vitro* antimicrobial evaluation of whole-plant extracts of *Eleucine indica.* J Med Plants Studies. 2017; 5(4):99-102.

73. Oyedemi BO, Oyedemi SO, Chibuzor JV, Ijeh IL, Coopoosamy RM, Aiyegoro AO. Pharmacological Evaluation of Selected Medicinal Plants Used in the Management of Oral and Skin Infections in Ebem-Ohafia District, Abia State, Nigeria. Hindawi. The Sci World J. 2011. Article ID 4757458, 16 https://doi.org/10.1155/2018/4757458

74. Ettebong EO, Etukakpan E, Bassey A. Anticonvulsant Potentials of Ethanolic Extract of *Eleusine indica.* Journal of Coastal Life Medicine. 2016; 4(12):965-968.

75. Garcia MTH, Malaguit CJA, Verte AJD, Cometa RP, Dimayacyac ME, Taganap ABP *et al*. Antiurolithiatic activity of ethanolic extract of Eleusine indica Linn. (Poaceae) leaves in ethylene glycol-induced urolithiasis in male Sprague Dawley rats. Asia Pacific J Allied Health Sci. 2018; 1:91-112.

76. Ettebong EO, Nwafor PA. Subchronic oral toxicity evaluation of ethanolic whole plant extract of *Eleucine indica* on haematological and biochemical indices in Wistar albino rats. J Coastal Life Med. 2016; 4(2):161-166.

77. Ettebong EO, Nwafor PA, Ubulom PE, Udobang JA. The toxicological assessment of ethanolic whole-plant extract of *Eleucine indica* in Wistar albino rats. J Herbmed Pharmacol. 2017; 6(2):49-54.