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Antibacterial and Pesticidal Potential of Parts (Root, Seed and Leave) of *Erythrophleum Suaveolens*

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Abstract

Erythrophleum suaveolens has been used in a number of places for treating several diseases. In the present study, the plant root, seed and leaves were screened for phytochemicals, bacterial and pesticidal properties using standard procedures. The results show the presence of tannins, terpenoids, saponnins, and alkaloids in the roots' methanol and hexane, seed and leaf aqueous extracts while cardiac glycosides were found in the roots and seed extracts, basalms was found present in the seed and leave extracts. The bioassay using modified Muella Hinton agar method indicated sensitivity of the bacterial (P. aeruginosa, S. aureus, E. coli and K. aerogenes) strains to the plant root extracts (methanol and hexane) and standard antibiotics (ciprofloxacin) with the methanol extract being more active. The bioassay of plant seed (100 %) and leave (52.5 %) against Bruchinae (Bruchid beetle) reveals the potential pesticidal activity. The E. suaveolens seed was found to be more bioactive.

Key words: Phytochemicals, antibacterial, pesticidal activity, *Erythrophleum suaveolens*,

Introduction

Medicinal plants have become a vital element in indigenous medicinal systems and a number of scientific investigations have highlighted the importance and contributions of many plant families (Kareem *et al*., 2010). They serve as therapeutic agents in prevention of many diseases and their medicinal benefits can be traced to specific compounds found in them (Sofowora *et al*., 2013, Babu *et al*., 2015; Boy *et al*., 2018).

The medicinal property of plants depends on the presence of a variety of chemical substances known as secondary metabolites. These secondary metabolites are constituents synthesized by plants in addition to the primary metabolites, which may be concentrated in different parts of the plants. Some of these compounds include saponins, glycosides,

flavonoids, alkaloids, steroids, terpenoids, tannins and volatile oils (Agber *et al*., 2019). These phytochemicals differ from one plant to another and are present at varying concentrations (Sofowora *et al*., 2013). The plant is a member of Caesalpinioideae family and therapeutic dose has been used in different locations for treating several diseases (Nyamukuru *et al*., 2017). It is a perennial tree that grows up to 30 m in height with often, low-branching and produces a dense spreading crown. It is natively called *obo* and *erun* (Yoruba), *inyi* (Igbo), *baska* (Hausa), *ene* (Idoma), k*or* (Tiv) and commonly referred to as sassy, sasswood, red water tree and ordeal tree in English. Studies have shown that *Erythrophleum* species are extremely toxic to livestock especially sheep and cow. Therefore, cattle herders are always very careful not to allow their animal to graze along the routes where the trees of these species are known to grow (Akinpelu *et al*., 2012).

Erythrophleum suaveolens is widely distributed in tropical Africa, from Senegal east to Sudan and South throughout Central Africa, Kenya, Tanzania and South Africa to Transvaal. The plant is used traditionally as arrow poison, for fishing, curtailing pest and taking of oath, among others (Ekhuemelo *et al*., 2019).

The bark is employed as a purgative and an emetic. An anthelmintic made from a diluted infusion of the roots is particularly effective against tapeworms. Bark extracts were utilized to treat heart failure in the West in the late 19th century. This technique was put to an end when digitoxine was discovered which showed more effectiveness in treating heart failures with less side effects. The crushed bark is utilized topically to treat filaria-related swellings. Headaches were treated by snuffing the powdered bark. To relieve generalized body discomfort, a decoction of the roots and bark was applied. Alkaloids, in particular, have been found to be one of the variety of medically useful compounds in the plant. An extract of the bark in water has an alkaloid concentration ranging from 0.3 - 1.5%, which varies depending on the age of the tree (Okeyo, 2008).

The bark and roots of *Erythrophleum suaveolens* are used as insecticide for killing larvae of *Anopheles gambiae* mosquitoes (Mnguu *et al*., 2015). Akinpelu *et al*. (2012) demonstrated the mulluscidal effects of ethanolic extract of the stem bark against freshwater snail, *Lanistes lybicus*. saponin was implicated for the effects which was dose dependent as mortality increased with relative increase in the saponin concentrations. The bark has been used as an ordeal poison in Tanzania, Malawi and Zimbabwe. Medicinal plants from this genus also bring positive results when employed as agents to invigorate and promote blood circulation, and as emetic drug, anesthetic, anthelmintic, anti-malaria, analgesic, disinfectant, dermatitis, convulsion, inflammation, cardiac problems, venom intoxication, headaches, oedemas, gangrenous wound, and rheumatism (Rice-Evans, *et al*., 1995). The timber of *Erythrophleum suoveolens* and several *other Erythrophleum* species is marked under the trade name "Missanda" it is used for furniture, heavy and light construction, posts, poles and tool handles. The wood is used as firewood and to make good-quality charcoal, used in iron working. The gum from the bark is used to make basket water proof and to fix arrow heads and axe handle. The folliage is reportedly used as a fodder but source from several other countries report that it is toxic and that cattle are kept away from it (Rice-Evans, *et al*., 1995). The compounds found in plants are of many kinds, but most are in four major biochemical classes; alkaloids, glycosides, polyphenols, and terpenes (Al-Kassien, 2009).

Ekhuemelo *et al*. (2019) assessed the antibacterial properties of sawdust and stem bark of *Erythrophleum suaveolens* extracts on selected wood bacteria. Methanol extracts were the most active extracts. Their study also showed presence of phytochemicals such as alkaloids, steroids, saponins, tannins, flavonoids, anthraquinones and glycosides. Ahmont *et al*. (2020) reported isolation of five new cassane-type diterpenoid heterosides, i.e. two cassane-type amides,

two erythrophlamine-type amine esters and a non-nitrogenous erythrophlamine analogue from the root barks and the seeds of *Erythrophleum suaveolens*.

The present study screened *Erytrophleum suaveolens* (root and stem bark, seed and leaves) for phytochemicals, antibacterial and pesticidal activities of the plant parts.

Plate 1: Pictorial representation of *Erytrophleum suaveolens*

Materials and Methods

Samples collection

Fresh root, seed and leaves of *Erytrophleum suaveolens* were collected from a rural village (Uga Adoka, Otukpo) in Benue state, Nigeria and identified by Mr. J.I. Waya of the Biological Science Department, Benue State University, Makurdi, Benue State.

The leaves, seeds (gently removed from its pods), root-bark (separated from the root) of *E. suaveolens* were washed and air-dried in shade for three weeks and then pulverized. The powdered samples were stored separately in plastic containers properly labeled for further analysis.

The powdered plant root bark (50 g) was macerated in 300 mL of 95 % methanol for 72 h. After which they were filtered and concentrated over a water bath (60 °C) to completely evaporate. This was repeated for n-hexane to give the methanol and hexane root extracts (Chira *et al.*, 2012). Furthermore, 20 g each of seed and leaf samples were macerated separately with 100 mL distilled water for 72 h. The samples were filtered and concentrated over water bath to obtain aqueous extract. The extracts were stored in airtight containers for further analysis (Chira *et al.*, 2012).

Phytochemical tests.

The extracts were assessed for the existence of the phytochemicals by using the following standard by Trease and Evans (2009)

Test for Saponins: 5.0 mL of extract was shaken vigorously to obtain a stable and persistent froth.

Test for Tannins: To 0.5 mL of the extract solution, 1 mL of distilled water and two drops of ferric chloride solution were added and observed for brownish green or a blue coloration confirming the presence of tannins.

Test for flavonoids: about 0.5 g of dried powdered plant sample was boiled in 10 mL ethanol and filtered. Few pieces of magnesium ribbon and few drops of concentrated HCl were carefully added to the filtrate. Red colour indicated the presence of flavonoids

Test for alkaloids: about 2 mL of 2 N HCl was added to 5 mL aqueous extract and the solution was heated with stirring in a water bath for 10 min. The cooled solution was filtered and a few drops of Dragendorff's reagent were added. Reddish-brown precipitate indicated the presence of alkaloids.

Test for steroids: about 0.5 g of dried powdered plant sample was mixed with 10 mL chloroform and filtered then added 1 mL acetic anhydride and few drops of concentrated H_2SO_4 to the filtrate. Red colouration at the interface of the miscible liquids indicated the presence of steroids.

Test for Terpenoids: a 5 mL of extract was mixed with 2 mL of chloroform in a test tube. Thereafter, 3 mL of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface reddish brown colouration was formed indicating the presence of terpenoids.

Test for Cardiac Glycosides: For cardiac glycosides, a 5 mL of extract was mixed with glacial acetic acid containing one drop of ferric chloride. The above mixture was carefully added to 1 mL of concentrated H_2SO_4. The presence of cardiac glycoside was detected by the formation of brown ring.

Test for Balsams: **a**bout 3 drops of alcoholic ferric chlorides solution where added to 0.5 mL of each extract a dim green shading demonstrates the nearness of balsams.

Culture and isolation of the bacteria

The following test microorganisms were used for the assessment, 1 gram-positive, and 3 gram-negative bacteria respectively: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella aerogenes*. The organisms were sub-cultured and inoculated twice to get a pure culture in nutrient agar while Mueller Hinton Agar (well-in-agar) method was used in antibiotic sensitivity testing (Akharaiyi *et al*., 2011).

Sensitivity test

Susceptibility of bacterial strains to the plant extracts and standard antibiotics was carried out following a modified bioassay method of Muella Hinton agar. The standard antibiotic used was ciprofloxacin (1 mg mL^{-1}) DMSO was also used as a control. Mueller Hinton sterile agar plates were seeded with 100 ìL of suspension of indicator bacterial strains containing approximately 10^5 cells and allowed to stand for 30 min at room temperature. Using a sterile cork borer of 5 mm in diameter, 5 wells were made on the four sets of seeded plates, 3 plates for each bacteria and these were filled with plant extracts of known concentrations and the standard antibiotics and DMSO. The plates were properly labeled and incubated at 37 °C for 24 hours and the zones of inhibition were measured at the end of the incubation period (Akharaiyi *et al*., 2011).

Bioassay for the pulverized seed and leave of *E. suaveolens*

The plant materials were assayed for pesticidal potency using the method described by Dharmasena *et al.* (2001). To assess the efficacy of the extracts as insecticide, bruchid beetles were confined in sterile plastic vessel (height 60 cm and diameter 25 cm). This was achieved by adding 0.01 g of the seed and 0.2 g of leave powder to the plastic vessels for 24 hours and then 40 bruchids were introduced into each plastic container covered with a net held with rubber band for ventilations. An empty plastic vessel served as control. The mortality of the weevils was assessed after 24 hours for 3 days.

The percentage mortality, was determined using the equation below Dharmasena *et al*. (2001

$$\% \text{ Mortality} = \frac{\text{number of dead insects}}{\text{number of initial insect}} \times 100 \qquad \text{Equation [1]}$$

Results and Discussion

The results of the qualitative phytochemical analysis, sensitivity tests and bioassay for pesticidal activity are presented in Table 1, 2 and 3.

Table 1: **The phytochemical constituents of extract of *E. suaveolens* root, seed and leave**

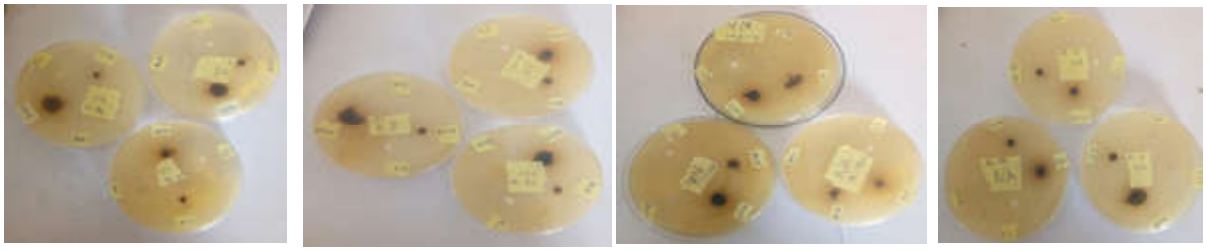
Phytochemicals	Root Extracts		Seed	Leave
	Methanol	Hexane	Water	Water
Tannins	+	+	+	+
Cardiac glycosides	+	+	+	-
Flavonoids	+	+	-	-
Terpenoids	+	+	+	+
Saponnins	+	+	+	+
Alkaloids	+	+	+	+
Balsams			+	+

Key: - absent, + present

Table 2: Zone of inhibition of the microbes with *E. suaveolens* root bark extracts

Bacteria	Extracts		Controls	
	Hexane (mm)	Methanol (mm)	DMSO (mm)	Ciprofloxacin (mm)
P. earuginosa	0.70	1.20	0.80	3.20
S. aureus	0.70	1.30	0.90	1.70
E. coli	0.60	1.00	0.60	1.70
K. earogenes	0.60	0.90	0.60	2.00

Key: 0 – zero Mortality



A B C D

Plate 2: A Plate showing sensitivity test for *P. aruginosa, E. coli, Klebsiella arogenes and S. aureus*

A: Sensitivity test for *P. aruginosa* (gram negative). B: Sensitivity test for *E. coli* (gram negative). C: Sensitivity test for *Klebsiella arogenes* (gram negative). D: Sensitivity test for *S. aureus* (gram positive).

Key: (hexane (n-H), hexane root extract, (Meth), methanol root extract (mR) and controls ciprofloxacin (Ci) and DMSO (Dm).

Table 1 gives a clear view of some phytochemical classes of compounds in the root bark of *E. suaveolens* which include: tannins, cardiac glycosides, flavonoids, terpenoids, saponnins, and alkaloids. The presence of alkaloids and saponins may be the reason for plant's antibacterial activity. Ekhuemelo *et al*. (2019) also reported the same phytochemicals especially for hexane and methanol root bark extracts of the plant.

E. suaveolens root bark extract showed anti-bacterial activity against all the microbes *(P. aruginosa, S. aureus, E. coli, Klebsiella arogenes)* with the zone of inhibition ranging from (0.6 -1.3 mm) which was greater than the zone of inhibition of the control (DMSO) (0.6- 0.9 mm) but inhibited lesser than control Ciprofloxacin (1- 3 mm) (Table 2). Methanol extract exhibited a zone of inhibition on the microbes ranging from (1.0-1.3 mm) and hexane extract showed zones of inhibition of (0.6- 0.7) on the microbes. The sensitivity tests using *E. suaveolens* root bark indicated that it can be useful anti-bacteria agent since it inhibited the growth of even resistant bacteria like *Staphyloccocus aureus*. Alkaloids possess a lot of pharmacological activities which includes antibacterial amongst others (Kareem, *et al*., 2010, Ogboru *et al*., 2015). Steroids also have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones. Phenolic compounds possess biological properties such as cardiovascular protection antiapoptosis, anti-inflammation, anti-aging, anti-atherosclerosis, anti-carcinogen, improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Babu, *et al*., 2015, Chira *et al*., 2012). In the same vein flavonoids account for pharmacological and biochemical actions viz., antioxidant, anti-allergic, antiinflammatory, hepatoprotective, anti-carcinogenic, anti-viral and anti-thrombotic activities. Tannins are used in antihemorrhoidal, hemostatic and anti-

diarrheal preparations. Saponins taste bitter and forms foams in aqueous solutions, can coagulate and precipitate red blood cells (hemolysis) and are helpful in lowering cholesterol (can bind to the cholesterol), as antioxidant and anti-inflammatory agents (Sabri *et al*., 2012 and Ogboru *et al*., 2015). Also, terpenoids have antibacterial properties and play curative role in healing lesions, reinforce the skin, and restore inflamed tissues by increasing blood supply (Samejo, *et al.,* 2013; Boy, *et al*., 2018)).

Bioassay of *E.suaveolens* seeds and leaves

The study revealed that the buchid beetles treated with *E. suaveolens* seeds and leaves (Table 3) gave promising levels of control over beetles at 24 -72 h. Beetles treated with 0.01 g of seed recorded 28 dead beetles within 24 h, another 8 beetles died in addition after 48 h and lastly 4 more beetles died at the end of 72 h resulting to 100 % mortality. Beetles treated with 0.2 g of the leaves recorded 8 buchid beetles dead after 24 h, next 6 more beetles died after 48 h, and lastly 7 beetles died after 72 h leading to 52.5 % mortality. Thus, the *E. suaveolens* seeds were found to be more potent against the beetles compared to the leaves. The reason is still open for further investigations. Terpenoids were reported by Don-Pedro (Ito and Ighere, 2017) to be a fumigant. The insect dies due to anorexia arising from drastic reduction in insect respiratory activities. Seeds and bark of *Erythrophleum suaveolens* contain cassane diterpenes (Ahmont, *et al*., 2020) which are alkaloids and are usually used for their defense but, may also be responsible for this pesticidal potential of the seeds. Yusuf *et al*, (2022) have also demonstrated pesticidal activity of stem bark extract of *E. suaveolens against Aspergillus flavus.* Other studies have proved that a group of C-32 or C-34 linear fatty acids called acetogenins is associated with the insecticidal activity (Dharmasena *et al*., 2001).

Conclusion

The qualitative phytochemical screening of *E. suaveolens* root extracts (methanol and hexane) revealed the presence of alkaloids, terpenoids, cardiac glycosides, saponins, tannins, flavonoids which may be responsible for the biological activity of the plant. The organisms (*S. aureus, P. aeruginosa, E. coli, K. aerogenes*) were sensitive to the root extracts (methanol and hexane) though, methanol extract was more active. The study suggests that *E. Suaveolens* possess pesticidal properties and can be used to control pests especially buchid beetles. It was found that the seed was more effective against the pests compared to the leaf. Hundred percent mortality was recorded for the seed at the end of 72 h. Hence, *E. suaveolens* seed can be used in the formulation bio-pesticides.

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