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*Full Length Research Paper*

# Foliar Epidermal Microscopy, Phytochemical Analysis and Antimicrobial activities of Leaves and Stem bark of *Erythrophleum suaveolens* Guill. & Perr. (Brenan) (Family: Fabaceae/Caesalpinioideae)

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***Abstract***
*The foliar epidermal microscopy, phytochemical analysis and anti-microbial activities of the leaves and stem bark of Erythrophleum suaveolens were carried out. The leaf epidermal microscopy revealed anomocytic stomata type on the lower surface. Trichomes were not observed on both surfaces. The phytochemical analysis revealed the presence of carbohydrates, flavonoids, steroids and alkaloids (hexane extract); saponin, tannin, terpenes, carbohydrates, flavonoids and steroids (ethyl-acetate extract); carbohydrates, terpenes, steroids and alkaloids (methanol extracts) of the stem bark. Absence of saponin, tannin, terpenes, phenols and anthraquinone (hexane extract); alkaloids, phenols and anthraqinones (ethyl-acetate extracts); saponin, tannins, phenols and anthraquinone (methanol extracts) on the stem bark. The leaves had tannin, flavonoids, steroid and phenols (ethyl-acetate extract); tannin, carbohydrates, flavonoids, terpenes, steroids and phenol (methanol extract). Thin layer chromatography of the leaf (hexane extract) showed three spots with Rf values of 0.2, 0.45 and 0.74. The HPLC spectrum of the leaf methanol extract showed twelve peaks. Peaks with retention times of 5.475 minutes, 7.542 minutes and 12.483 minutes corresponded to caffeic acid, rutin and ferulic acid respectively. The anti-microbial screening was carried out on the successive extract of the leaves and stem bark. The extracts were screened against two standard strains of American Typed Culture Collection of Salmonella paratyphi and Candida albicans and five clinical isolates of Pseudomonas aeroginosa, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae and Escherichia coli, using agar dilution method. At the concentration of 2mg/ml, the foliar hexane and methanol extracts did not exhibit any anti-microbial activity against the test micro-organisms but the ethyl-acetate extract showed activity against all test organisms. The stem barks extracts showed no activity on the test organisms except the methanol extract which exhibited activity on Pseudomonas aeroginosa. Amoxicillin used as standard, inhibited the growth of all the test micro-organisms. The results from the study provide finger print for the identification of Erythrophleum suaveolens.*

***Key words**: Erythrophleum suaveolens, microscopy, phytochemistry, antimicrobial*

## Introduction

Medicinal plant research has been and continues to be considered as a fruitful approach for the search of new drugs especially as the consequence of loss of biodiversity in same context has posed great challenges to the third world continues. The search of herbal preparations that do not produce any adverse effect in the non-target organisms, and which are easily biodegraded remains a challenge to research issues for scientists (WHO, 1985). *Erytrophleum suaveolens* (Guill. & Perr.) Brenan. (Syn. *Erythrophleum guineense*) belongs to the family Fabaceae/Leguninosae and the sub-family Caesalpinoideae. *Erythrophleum suaveolens* also known as sassy,

sasswood, red water tree or ordeal plant is distributed from Senegal, Sudan, Kenya, Mozambique, Nigeria and Zimbabwe; it has been introduced as ornamental in tropical Asia. *Erythrophleum suaveolens* comprises of 10 species, 5 occur in continental Africa, 1 in Madagascar, 3 in Eastern Asia and 1 in Australia (Okeyo, 2006). Three species are found in Nigeria these are: *E. africanum* Harms; *E. ivorense* A. Chev. and *E. suaveolens* (Guill. & Perr.) Brenan. They are mostly trees considered to be poisonous to livestock especially sheep and cow, though the bark and leaves are used medicinally. In the savanna regions, the cattle herders are very careful not to allow their animals to graze along the route where the trees of these species are known to grow (Aiyegoro *et al.,* 2007).

The powdered roots are used for leprous and syphilitic ulcers (Irvine, 1961). Bark decoctions are used as a lotion for parasites. The powdered bark is given as snuff for headaches and sinusitis (Irvine, 1961). The chemical composition of the bark varies, and sometimes the bark is reddish, pinkish or whitish. Yellow or pinkish barked forms were reserved for ordeals, while the whitish barked forms which are poorer in tannin are more poisonous were used for arrow and hunting poisons (Irvine, 1961). It is believed that the different amount of poison in the barks of *E. ivorensis* and *E. guineense* depends on the tannin content which is higher in the latter. Though chemical study of the *Erythrophleum* species is by no means complete, many alkaloids and their derivatives have been obtained, well crystalized, though their exact composition is not yet determined. An alkaloid *ethryrophleine* was isolated from the bark along with tannin substances (Irvine 1961). Four alkaloids: *cassaine, cassaidine, norcassaidine* and *homophleine,* were isolated and characterized (Ruzica *et al*. 1983) *E. guineense* and *E. ivorense,* exhibited great variability in their alkaloids content. Tannin, a saponin, a phytosterol and a flavonoside were isolated (Dussy, 1959). The plant has been reported to contain alkaloids, cassamidines and erythrophleine. The alkaloids found have similar pharmacological activities as digitoxine and ouabain. The alkaloid content of the bark ranged from 0.3% to 1.5% (Okeyo, 2006). An acid alcohol, pinitol and wax containing a large proportion of *hexacosanol* have been reported (Irvine, 1961).

The poison in the species of *Erythrophleum* acts as a local anesthetic and as a cardiac poison. Rigal showed that the bark's poisonous character was proportional to the alkaloid content, but that it is always greater than that of the alkaloids themselves; moreover, that *E. ivorense* is less poisonous than *E. guineense*, thus confirming a statement made in 1905, that the action of *erythrophleine* on the circulation resembles that of digitalis in raising blood pressure and slowing pulse and increasing the force of the heart's action. It decreases respiration and in overdose there are symptoms of depression of the circulation, difficulty in breathing, vomiting and convulsion, the latter resulting from direct action of the medulla center. Though not adopted so far as a medicine for heart disease, it is said to be of use in spasmodic asthma. It is a local anaesthetic like cocaine, but is more powerful and lasting (Irvine, 1961), though the hydrochloride has been recommended for use in dental surgery (Irvine, 1961). Elevation of activities of acid and alkaline phosphate in the intestine and hepatopancreas, haemolynph and total protein level were observed in tissues of fresh water snail in an investigation of the activity of saponin from ethanolic extract of *E. suaveolens*; crude, pure, aqueous and lipid fraction extract of air-dried leaves of *E. suaveolens* is not recommended for use on the fingerlings of the clarid catfish despite its anaesthetic effect (Akinpelu *et al*., 2012). It is also reported to be used as poison or repellant against rodents, insects and some aquatic animals and also in tanning hides and as dye (Akinpelu *et al*., 2012).

The main parts of *Erythrophleum suaveolens* harvested for medicinal purposes are roots and bark, while the roots are exploited for timber (Okeyo, 2006). A cold infusion of the bark is sometimes used as an ordinary emetic and purgative (Burkill, 1995). It is strongly astringent, causes irritation to the eye, but in collyrium acts as anaestehetic (Burkill, 1995). It is considered anthelmintic. Small portions are taken for malaria. A decoction is used in Gabon on gangrenous ulcers, skin complaints, inflammations and sore on the sole of the feet. In Congo, a decoction of the bark is made into mouth wash and gargle for throat, mouth and tooth affections. It is applied as a wash in cases of chicken pox and cases of skin diseases, oedemas, gangrenous wounds and rheumatism and arthritis, and in casamance to expel guinea worm. It is added to palm-wine to increase its potency (Burkill, 1995). In West Africa the powdered bark is mixed with the residue of palm oil processing, and after boiling it is mixed with seeds of maize, cowpea or cotton which effectively reduces pest damage to the seeds (Neuwinger, 1996). The bark is used for tanning hides. The leaves are poisonous to horses and cattle. They are placed among stored corn to keep away insect pests. The seed-pods and seeds are poisonous to sheep. Curiously in Uganda the fruit is a favorite food of elephants that are reported to be responsible for dispersing the seeds. The elephants must thus eat the fruits without crushing the seeds and so avoid poisoning themselves. The seed is in fact recorded as being more toxic than the bark in spite of having, in general, lower alkaloid content, allegedly due to the simultaneous presence of a strongly haemolytic saponin acting synergistically (Burkill, 1995).

Dongmo *et al*., 2001, carried out the anti-inflammatory and analgesic properties of the stem bark of the plant, Aiyegoro *et al.,* 2007 researched on the in-vitro anti-bacterial on the stem bark of the plant and Ogundeko *et al.,* 2014 conducted the comparative effect of the cold hydro stem bark extracts of the plant on gastrointestinal muscle of rabbit Jejunum. However, the microscopic profile of the leaf is not available in literature. This information would be useful in the identification of the plant as a crude drug.
Local name of the plant include: Gwaska (Hausa), Orachi (Igbo) and Erun obo (Yoruba).

**Materials and methods**

*Plant Collection*

The leaves and stem bark of *Erythrophleum suaveolens* were collected from Zungeru, Niger State in May, 2013. A herbarium specimen (NIPRD/H/6612) was deposited at NIPRD herbarium.

*Plant Preparation*

The plant leaves and stem bark were dried at room temperature (27 ± 1$^{o}$C) and then powdered using a mortar and pestle. The powdered samples were used for the phytochemical and HPLC screening. Screening of the filtrate for possible phytochemicals was carried out as described by Tiwari *et al.,* 2011; Harborne, 1973; Trease and Evans, 1989 and Sofowora, 2008.

*Foliar Epidermal Microscopy*

The dried leaf was used for the microscopy and the method used by Ugbabe and Ayodele (2008) was adopted. About 5mm – 1cm squared leaf fragments were obtained from the standard median portion of the leaf and macerated in concentrated Nitric acid in petri-dish for a period of 24 hrs. The appearance of bubbles on the surface of the leaf fragment indicated their suitability for separation. The fragments were transferred into water in a petri-dish with a pair of forceps. Both epidermises were carefully separated by teasing them apart and pulling each epidermis back at itself. The leaf epidermises were cleaned with the Carmel hair brush. These were rinsed in distilled water and later transferred into 50% ethanol to harden. They were then stained in Safranin O for 5 minutes and excess stain washed off in water. They were then mounted in glycerine on a slide with the edge of the cover slips ringed with nail vanish to prevent dehydration. The slides were labeled appropriately and examined under the light microscope while photographs were taken using NICON AFX-DX microscope with NICON FX-35DX camera attached at a magnification of x100 and x400.

*Phytochemical screening*

Phytochemical screening for various constituents such as carbobydrates, anthraquinone, saponin, tannin, flavonoids, sterols, phenols, terpenes and alkaloids was carried out on the powdered leaf and stem bark samples using standared methods: (Evans, 2002, Sofowora, 1993, Brain and Turner, 1975, Segelman *et al*., 1971). Each of the tests was qualitatively expressed as negative (-) or positive (+).

*Test for carbohydrates (General Test-Molisch's Test)*

A few drops of Molisch's reagent was added to 2mL of the water extract, small quantity of conc. Sulphuric acid was also added and allowed to form a lower layer. A purple layer on the surface of the liquid indicated the presence of Carbohydrate. Each mixture was shaken and allowed to stand for 10 minutes and distilled with 5mL of water, a purple precipitate indicated the presence of Carbohydrates.

*Test for Alkaloids*

Wagner's Test:

The filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

*Test for Saponins*

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

*Test for Phenols*

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

*Test for Tannins:*

2 ml of extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

*Test for Flavonoids:*

Lead acetate Test: The extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

*Test for Anthraquinones*

Borntrager's test: 5 ml of chloroform was added into 2 ml of extract and the mixture shaken for about 5 minutes. It was filtered and the filtrate shaken with equal volume of 10 % ammonia solution. A pink-violet or red colour in the ammoniacal layer (lower layer) indicates the presence of anthraquinones.

*Test for Terpenoids*

Salkowski test: 2 ml of the extract was mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate the presence of terpenoids.

*Test for Steroids*

2 ml of acetic anhydride was added to 2 ml of the extract followed by 2 ml of $H_2SO_4$. Color change from violet to blue, or green in some samples indicate the presence of steroids.

*Thin Layer Chromatography Analysis*

Thin layer chromatography of the hexane extract was performed on normal phase silica gel precoated on glass. The mobile phase consisted of hexane and ethyl acetate (3:1). Reference standard β-sitosterol was spotted alongside the extract as control. The developed plate was air-dried, viewed in day light, under UV at 366 nm and in iodine vapor tank. The Rf values of the constituents were determined.

*High Performance Liquid Chromatography Analysis*

The chromatographic system includes Shimadzu HPLC system consisting of Ultra-Fast LC-20AB prominence equipped with SIL-20AC auto-sampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector (UV-DAD); column oven CTO-20AC, system controller CBM- 20Alite and Windows LCsolution software (Shimadzu Corporation, Kyoto Japan); column, VP-ODS 5µm and dimensions (150 x 4.6 mm). The chromatographic conditions included mobile phase solvent A: 0.2% v/v formic acid and solvent B: acetonitrile; elution mode was isocratic; flow rate 0.6 ml/min; injection volume 5 µl of 50 mg/ml solution of *Erythrophleum suaveolens* leaf methanol straight run extract dissolved in methanol; detection was at UV 254 nm wavelength. Reference standards, rutin, quercetin, caffeic acid, ferulic acid and apigenin (Fluka, Germany) 50 µg/ml in methanol were analyzed separately under the same condition as the extract. The HPLC operating conditions were programmed to give the following: solvent B: 20% and column oven temperature 40$^{o}$C. The total run time was 40 minutes (Okhale *et al.,* 2016)

*Anti-microbial screening*

Preparation of extracts: The powdered leaf and stem bark were macerated successively using hexane, ethyl-acetate and methanol at room temperature (28 ± 2$^{o}$C) for 24hrs. (Sofowora,2008). The extracts were filtered and the filtrate concentrated in *vacuo* using a rotary evaporator at 45$^{o}$C. The extracts were kept in sealed containers until required.

Preparation of the test organisms: A wire loop-full (wire loop flamed red hot and cooled) of each test organism was taken aseptically from their respective slants and sub-cultured into Mac-Cartney bottles containing 5mL of freshly prepared nutrient broth and placed in the incubator for 24hrs. at 37$^{o}$C. The 24 hour culture was sub-cultured using a wire loop-full into freshly prepared broth and incubated at 37$^{o}$C for 3hrs. (Containing approximately 1.25 x $10^6$ – 1.25 x $10^7$ colony forming units). This is equivalent to half McFarland standard.

Screening against micro-organisms: The extracts (successful extracts of Hexane, Ethyl-acetate and Methanol) were screened for activity against *Salmonella paratyphi* (ATCC 69150) and *Candida albicans* (ATCC 22015) and clinical isolates of *Pseudomonas aeroginosa, Staphilococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiells pneumoniae* and *Candida albicans* using agar dilution method (Mitscher *et al.,* 1972). 0.5 mL of Dimethylsulphoxide (DMSO) or acetone was used to dissolve the crude extract and made up to 1mL with sterile water. 1 ml each of the crude extract (containing 32mg) was introduced into 15mL of molten nutrient agar placed in water at 45$^{o}$C. These were mixed properly and poured into sterile petri-dishes to give final concentration of 2mg/ml. The dishes which were prepared in duplicates and then allowed to gel and thereafter, the test organisms were inoculated by streaking into the nutrient agar using a wire loop which was left to stand for 15 minutes. Control plates were also set containing only agar and test organisms (organism viability control), plates containing agar and DMSO or acetone and plates containing agar and sterile water, also served as controls. The standard Amoxicillin, at a final concentration of 2mg/ml was treated the same way as the crude extracts. The petri-dishes were incubated overnight at 37$^{o}$C (20 - 24hrs.) after which they were observed for microbial growth inhibition. All procedures were done aseptically in the biosafety cabinet to avoid the introduction of unwanted micro-organism from the environment.

**Results**

*Foliar Epidermal Microscopy*

The leaf epidermal microscopy revealed anomocytic stomata type on the lower epidermis while the upper layer had no stomata. The cell walls on the upper surface are wavier than on the lower surface. Trichomes were not observed on both leaf surfaces of the plant (Plate 1).

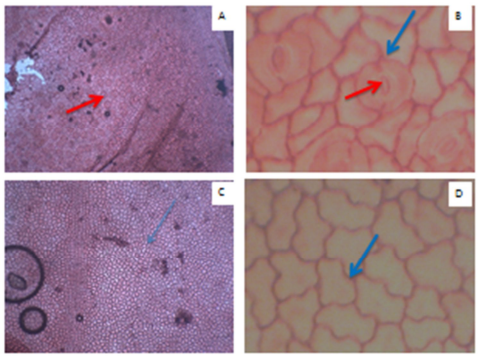

**Plate 1: Photomicrographs of the Leaf epidermis of *Erythrophleum suaveolens***
(A) Lower surface x 100 with numerous stomata
(B) Lower surface x400 with anomocytic stomata (red arrow)
(C) Upper surface x100 with no stomata and wavy cell walls
(D) Upper surface x400 with wavy cell walls (blue arrow) and no stomata

*Phytochemical screening*
The phytochemical screening of the stem bark and leaves for the various extracts are presented in Tables 1 and 2 respectively.

**Table 1.** Phytochemical screening of the stem bark of *Erythrophleum suaveolens*

| Constituent | Hexane extract | Ethyl-acetate extract | Methanol extract |
|---|---|---|---|
| **Saponin** | - | - | + |
| **Tannins** | - | - | - |
| **Carbohydrates** | + | + | + |
| **Flavonoids** | + | + | - |
| **Terpenes** | + | + | + |
| **Steroids** | + | + | + |
| **Alkaloids** | + | - | + |
| **Antraquinone** | - | - | - |
| **Phenols** | - | - | - |

***Key: -*** *= absence; + = presence*

**Table 2.** Phytochemical screening of the leaves of *Erythrophleum suaveolens*

| Constituent | Hexane extract | Ethyl-acetate extract | Methanol extract | Crude |
|---|---|---|---|---|
| **Saponin** | - | - | - | - |
| **Tannins** | - | + | + | + |
| **Carbohydrates** | - | - | + | + |
| **Flavonoids** | - | + | + | + |
| **Terpenes** | - | - | + | + |
| **Steroids** | - | + | + | + |
| **Alkaloids** | - | - | - | - |
| **Antraquinone** | - | - | - | - |
| **Phenols** | - | + | + | + |

***Key: -*** *= absence; + = presence*

*Thin Layer Chromatography Analysis*
Thin layer chromatography of *Erythrophleum suaveolens* leaf hexane extract showed three spots with $R_f$ values of 0.2, 0.45 and 0.74. β-sitosterol was used as reference and had $R_f$ value of 0.54. The phytoconstituents with $R_f$ values of 0.2 and 0.74 were yellow in day light and iodine vapor. The major constituent with $R_f$ value of 0.45 showed characteristic green colour in day light and pink colour under UV 366 nm. The reference β-sitosterol was detected only in iodine vapor. The *Erythrophleum suaveolens* leaf hexane extract did not contain β-sitosterol.

*High Performance Liquid Chromatography Analysis*

The HPLC spectrum showed twelve peaks with retention times of 3.677, 4.248, 4.865, 5.475, 6.174, 7.542, 12.483, 13.844, 16.274, 19.783, 37.888 and 40.015 minutes. Peaks with retention times of 5.475 minutes, 7.542 minutes and 12.483 minutes corresponded to caffeic acid, rutin and ferulic acid respectively (Figure 1).

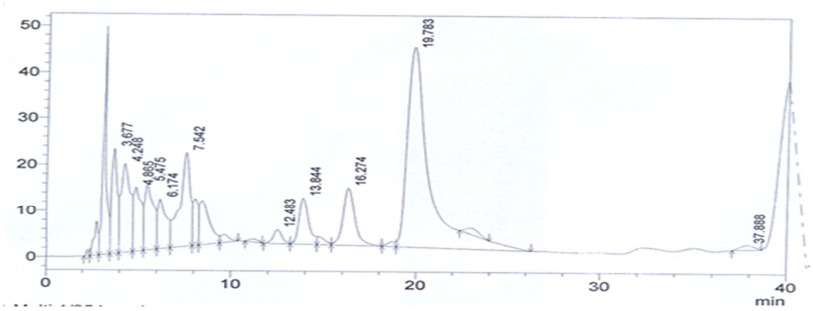


**Fig 1:** High Performance Liquid Chromatogram of *Erythrophleum suaveolens* leaf

*Anti-microbial screening*

The anti-microbial screening of the extracts of the leaf and stem-bark of *E. suaveolens* is presented in Tables 3 – 5. The test organisms are: *Staphilococcus aureus, Salmonella paratyphi* (ATCC 69150)*, Bacillus subtilis, Pseudomonas aeroginosa, Candida albicans* (ATCC 22015)*, Klebsiella pneumonia and Escherichia coli.* The screening against micro-organisms was done using agar dilution method (Mitscher *et al.,* 1972). The hexane extracts of the leaf and stem bark had no activities on all the test organisms (Table 3). The ethyl-acetate extract of the leaf had activity on all the test organisms while the extract of the stem-bark had no activity on all the test organisms (Table 4). The methanol extracts of the leaf had activity on *P. aeroginosa* but no activity on the other organisms (Table 5) while the methanol extract of the stem-bark had no activity on any of the test organisms used in this study. All the control plates did not show any inhibition of the growth of all the test organisms (Tables 3, 4, 5). Amoxicillin, used as standard, inhibited the growth of all the test organisms (Tables 3, 4, 5).

**Table 3.** Anti-microbial screening of the hexane extract of the leaf and stem bark of *Erythrophleum suaveolens*

| Parameters | Sa | St | Bs | Ps | Ca | Kp | Ec |
|---|---|---|---|---|---|---|---|
| **Stem bark** | - | - | - | - | - | - | - |
| **Leaf** | - | - | - | - | - | - | - |
| **Agar (Control)** | - | - | - | - | - | - | - |
| **DMSO (Control)** | - | - | - | - | - | - | - |
| **Acetone (Control)** | - | - | - | - | - | - | - |
| **Amoxicillin** | + | + | + | + | + | + | + |

***Key:***

*Sa = Staphilococcus aureus* *Ps = Pseudomonas aeroginosa* *Ec = Escherichia coli*
*St = Salmonella typhii* *Ca = Candida albicans* *+ = Activity*
*Bs = Bacillus subtilis* *Kp = Klebsiella pneumoniae* *- = No activity*

**Table 4.** Anti-microbial Screening of the Ethylacetate extract of the Leaf and Stem bark of *Erythrophleum suaveolens*

| Parameters | Sa | St | Bs | Ps | Ca | Kp | Ec |
|---|---|---|---|---|---|---|---|
| **Stem bark** | - | - | - | - | - | - | - |
| **Leaf** | + | + | + | + | + | + | + |
| **Agar (Control)** | - | - | - | - | - | - | - |
| **DMSO (Control)** | - | - | - | - | - | - | - |
| **Acetone (Control)** | - | - | - | - | - | - | - |
| **Amoxicillin** | + | + | + | + | + | + | + |

**Key:**

Sa = *Staphilococcus aureus* Ps = *Pseudomonas aeroginosa* Ec = *Escherichia coli*
St = *Salmonella typhii* Ca = *Candida albicans* + = Activity
Bs = *Bacillus subtilis* Kp = *Klebsiella pneumoniae* - = No activity

**Table 5.** Anti-microbial screening of the methanol extract of the leaf and stem bark of *Erythrophleum suaveolens*

| Parameters | Sa | St | Bs | Ps | Ca | Kp | Ec |
|---|---|---|---|---|---|---|---|
| **Stem bark** | - | - | - | - | - | - | - |
| **Leaf** | - | - | - | + | - | - | - |
| **Agar (Control)** | - | - | - | - | - | - | - |
| **DMSO (Control)** | - | - | - | - | - | - | - |
| **Acetone (Control)** | - | - | - | - | - | - | - |
| **Amoxicillin** | + | + | + | + | + | + | + |

***Key:***
*Sa = Staphilococcus aureus*
*St = Salmonella typhii*
*Bs = Bacillus subtilis*
*Ps = Pseudomonas aeroginosa*
*Ca = Candida albicans*
*Kp = Klebsiella pneumoniae*
*Ec = Escherichia coli*
*+ = Activity*
*- = No activity*

**Discussion**

The species is hypostomatic with stomata restricted to the lower surfaces of the leaves. The stomata type observed in the species studied is anomocytic. A stoma (*pl.* stomata) is a microscopic pore on the surface (epidermis) of land plants. It is surrounded by a pair of specialized epidermal cells called guard cells, which act as a turgor-driven valve that open and close the pores in response to given environmental conditions. Stomata act as a gateway for efficient gas exchange and water movement from the roots through the vasculature to the atmosphere. Transpiration via stomata supplies water and minerals to the entire plant system (Raven 2002).

The phytochemical analysis revealed the presence of carbohydrates, flavonoids, steroids and alkaloids in the hexane extract; saponin, tannin, terpenes, carbohydrates, flavonoids and steroids in the ethyl-acetate extract; carbohydrates, terpenes, steroids and alkaloids in the methanol extracts of the stem bark. And absence of saponin, tannin, terpenes, phenols and anthraquinone in the hexane extract; alkaloids, phenols and anthraqinones in the ethyl-acetate extracts; saponin, tannins, phenols and anthraquinone in the methanol extracts of the stem bark. While in the leaves, the presence of tannin, flavonoids, steroid and phenols in the ethyl-acetate extract; tannin, carbohydrates, flavonoids, terpenes, steroids and phenol in the methanol extract; all the parameters tested for in the hexane extracts of the leaves were absent.

The anti-microbial screening of the extracts of the leaf and stem-bark of *E. suaveolens* is presented in Tables 3-5. The test organisms are: *Staphilococcus aureus, Salmonella paratyphi* (ATCC 69150)*, Bacillus subtilis, Pseudomonas aeroginosa, Candida albicans* (ATCC 22015)*, Klebsiella pneumonia and Escherichia coli.* The screening against micro-organisms was done using agar dilution method (Mitscher *et al.,* 1972). The hexane extracts of the leaf and stem bark had no activities on all the test organisms. The ethyl-acetate extract of the leaf had activity on all the test organisms while the extract of the stem-bark had no activity on all the test organisms. The methanol extracts of the leaf had activity on *P. aeroginosa* but no activity on the other organisms while the methanol extract of the stem-bark had no activity on any of the test organisms used in this study. All the control plates did not show any inhibition of the growth of all the test organisms. Amoxicillin, used as standard, inhibited the growth of all the test organisms. *E. suaveolens* saponins could be used to prevent damage caused by free radicals and infections caused by pathogenic bacteria. In addition, 70:30 *E. suaveolens* saponin fraction stem bark could be a useful starting point if the active saponin principles are to be exploited for development into antimicrobial chemotherapeutic agents in line with the ongoing search for substances to replace the antibiotics in current clinical use which because of the emergence and spread of resistant organisms are less useful than before (Iwata, 1992; Chopra *et al*., 1997).

Thin layer chromatography of *Erythrophleum suaveolens* leaf hexane extract showed three spots with Rf values of 0.2, 0.45 and 0.74. β-sitosterol was used as reference and had Rf value of 0.54. The phytoconstituents with Rf values of 0.2 and 0.74 were yellow in day light and iodine vapor. The major constituent with Rf value of 0.45 showed characteristic green colour in day light and pink colour under UV 366 nm. The reference β-sitosterol was detected only in iodine vapor. The *Erythrophleum suaveolens* leaf hexane extract did not contain β-sitosterol.

The HPLC spectrum showed twelve peaks with retention times of 3.677, 4.248, 4.865, 5.475, 6.174, 7.542, 12.483, 13.844, 16.274, 19.783, 37.888 and 40.015 minutes. Peaks with retention times of 5.475 minutes, 7.542 minutes and 12.483 minutes corresponded to caffeic acid, rutin and ferulic acid respectively.

**Conclusion**

The ethyl-acetate extract of the leaf of *Erythrophleum suaveolens* exhibited significant broad spectrum anti-microbial activity against all the seven organisms (both Gram positive and Gram negative) used in this study. This activity could be compared with the activity of amoxicillin used as standard. Activity at the concentration of 2mg/ml is a promising result because further works could be done on the ethyl-acetate and methanol extracts of the leaf of *Erythrophleum suaveolens* which may lead to the development of antibiotic agent. The pharmacological activity of the extracts is being tested and will form part of another paper

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