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Vol. 10(39), pp. 686-694, 17 October, 2016
DOI: 10.5897/JMPR2016.6070
Article Number: 2DDB8D761340
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

*Full Length Research Paper*

# Stem bark extracts of *Erythrina excelsa* (BAK) and their biological activities

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Received 26 January, 2016; Accepted 6 May, 2016

**The species is known from Southern Nyanza, Migori in Kenya. The extraction of the stem bark was done sequentially using organic solvents starting with the least polar; n-hexane then dichloromethane, ethyl acetate and finally the most polar methanol. Antibacterial and antifungal activities of the crude extract and that of isolated compounds from the stem bark of *E. excelsa* were investigated. The crude extracts had substantial activity against the tested micro-organisms. Methanol extract was highly active with inhibition zones of 15 mm against *S. aureus* and 14 mm against both *B. subtillis* and *E. coli*. Ethyl acetate and dichloromethane had mild activity. In antifungal test, methanol extract had highest activity of 15 mm against *A. niger* and 13 mm against *C. albicans.* Dichloromethane extract was also active with inhibition zones of 14 mm against *A. niger* and 12 mm against *C. albicans* while ethyl acetate had mild activity. A total of five compounds were isolated; glutinosalactone A (1), glutinosalactone B (2), lupinifolin (3), sitosterol (4) and 3β-stigimasterol (5). The compounds were active against the bacterial and fungal test strains used. Glutinosalactone A (1) had an activity of 15 mm against A. *niger*, 11 mm against both *B. subtillis* and S. *aureas*. Glutinosalactone B (2) had a high activity of 11 mm in both B. *subtillis* and C. *albicans*. and lupinifolin (3) had mild activity of 9 mm against both A. *niger* and C. *albicans.* Stigimasterol and sitosterol had mild activity of 8 mm against A. *niger*.**

**Key words:** *Erythrina excelsa*, biological activities, ethnobotany, metabolites.

## INTRODUCTION

Medicinal plants use in the world as phytotherapy is old as humankind. Methods of use are, however, regionally variable. The usages of plants are further influenced by cultural characteristics of the population, flora, and environmental factors (Murray and Pizzarno, 1995). *Erythrina excelsa* is one of the numerous species in the genus *Erythrina.* There is over 130 species of "coral tree" that belong to the genus *Erythrina* which has been widely studied and are distributed in tropical and subtropical regions of the world. (Hickey and King, 1981). *E. excelsa* is usually found in riverine and swampy forest up to 1500 m altitude. The species is known from Kisii, Migori

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and parts of Kisumu in Kenya. The leaves and the root decoction of the species *E. secluxii* are used in the treatment of malaria (Gessler et al., 1995). The stem bark of *E. abysinicca* is used to treat trachoma and syphilis while its root bark is used to treat malaria and colic (Ichimaru et al., 1996). Extracts of plants from this genus have proven highly bioactive (Normark and Normark, 2002); *E. glauca* and *E. lysistemon* have been reported to poses antiviral, antibacterial and estrogenic activity (Ito, 1999). Aqueous extract of the stem bark of *E. senegalensis* have proved to have analgesic and anti-inflammatory effects (Saidu et al., 2000). In addition, the leaf or bark decoction or tincture from *E. mulungu* is considered to calm agitation and other disorders of the nervous system, including insomnia (Vasconcelos et al., 2007). Several uses have been reported. Table 1 shows medicinal uses of the various parts of *E. excelsa* in East Africa.

Analysis of various parts of the plants from this genus has demonstrated the presence of flavonoids, especially, isoflavones, pterocarpanes, flavanones and isoflavanones (Chacha et al., 2005). Terpenes have also been isolated from the same genus (Nkengfack et al., 1997). Biologically active alkaloids are also found in a number of species of the genus. Many of the alkaloids have been reported to have anti-inflammatory, cardioactive, narcotic and sedative activities (Palframan and Parsons, 2010).

*Erythrina burana* is an endemic species in Ethiopia, where, it is widely distributed (Hanelt et al*.,* 2001). It is also cultivated across its natural distribution range; and in Kenya and Ethiopia as a shade tree for coffee plantations. The species is also used as fodder for livestock (Hanelt et al*.,* 2001). The bark sap of *E. excelsa* is administered as an anti-dote for snake bites (Owuor et al., 2005). Its stem and root decoction is used in treatment of arthritis. This study was aimed at investigating the bioactivity of the crude extract and that of pure compounds obtained from the stem bark of *E. excelsa* to substantiate its usage as anti-dote for snake bites as well as in treatment of arthritis.

## MATERIALS AND METHODS

### Plant materials

The stem bark of *E. excelsa* was collected from Migori County in February 2011 and taken to Plant Science Laboratory of Kenyatta University for identification. The plant was authenticated as *E. excelsa*, Voucher specimen NMO/KU/EE/SB/001 by Mr. Lucas Karimi of the Department of Pharmacy and Complementary Medicine of Kenyatta University. Voucher specimens of the plant were also taken to Kenyatta University Herbarium where they were held for future reference. The sample was air dried under a shade for one month to a moisture index of 13% and ground into fine powder to pass through 0.5 mm sieve using a manual grinder.

### General extraction

The extraction of the stem bark was done sequentially using organic solvents starting with the least polar; n-hexane then dichloro-methane, ethyl acetate and finally the most polar methanol. 5 Kg of the air dried fine powdered sample was soaked in 4 L of n-hexane for 48 h then filtered and the filtrate concentrated under pressure using a rotary evaporator to obtain the hexane extract. The extraction was repeated once with n-hexane. The same procedure was followed replacing n-hexane with dichloromethane, ethyl acetate and then methanol in turn to obtain dichloromethane, ethyl acetate and methanol extracts. The extracts were the stored in a deep freezer.

### Isolation of compounds

#### *Methanol extract*

Column chromatography was used to fractionate the extracts. The methanol extract (30 g) was fractionated using solvents of increasing polarity starting with n-hexane to methanol. 500 ml of the solvent system was used in each case. This gave a total of 21 fractions. Fraction 72 to 79 (dichloromethane: methanol = 9:1) was subjected to preparative TLC using 100% DCM yielding two compounds. 19.7 mg of white crystals of compound **1** and 17.5 mg of white crystals of compound 2.

#### *DCM-hexane extract*

The DCM-hexane extract weighed a total of 53.0 g. 50.0 g was adsorbed in 5.0 g of silica gel and subjected to column chromatography (diameter 4.0 cm and length 60 cm) over a column of silica gel 240 G using solvents of increasing polarities from hexane to methanol. 500 ml of each solvent system was used in each case. This yielded a total of 18 fractions. Fractions 36 to 54 (hexane: DCM = 2:8) was subjected to a Sephadex column (DCM: MeOH = 50:50) yielding seven sub-fractions. Sub-fraction 20 to 24 was subjected to preparative TLC using 100% DCM and this resulted in 18.0 mg of yellow crystalline solid of **3** preparative TLC was carried out on fraction sub-fraction 10 to 19 using 100% DCM yielding two compounds; 14 mg of white crystals **4** and 12 mg of white crystals **5**.

### Spetral determination

Both one and two dimensional NMR spectra of compound 1 and 2 was done from the University of Kwazulu-Natal in South Africa using deuterated methanol as a solvent. The spectra for compound 3, 4 and 5 were determined from the University of Nairobi using chloroform. 15 mg of each the compounds; 1, 2, 3, 4 and 5 was packed in sample vial and taken for analysis.

### Bioassays: Antibacterial and antifungal activities

The bioassays were done at the Microbiology Laboratory of Kenyatta University to determine the bioactivities of the crude extracts and that of the isolated pure compounds. Nutrient agar (NA) was used for antibacterial tests and potato dextrose agar (PDA) for antifungal tests. Petri-dish plates containing lids were used. All the microbial organisms were obtained from the Department. These were; *Staphylococcus aureus* (ATCC 35844) and *Bacillus subtillis* (ATCC 6051) that were Gram-positive and *Escherichia coli* (ATCC 11775) that was Gram-negative. Filter paper disc assay method was employed (Chhabra and Uiso, 1991) in which filter paper discs (6 mm) impregnated with hexane, dichloromethane, ethyl acetate and methanol extracts was applied on plates containing a cultured micro-organism. The plates were then incubated at 37°C for 24 h, and then zones of inhibition

**Table 1.** Medicinal uses of various species of *Erythrina.*

| Use | Part utilized | Kind of extract/ way of administration | Species | Country | References |
|---|---|---|---|---|---|
| Trachoma | Bark | Unspecified, oral | *E. abyssinica* | | |
| Malaria | Roots | Unspecified, oral | *E. abyssinica* | Kenya | Ichimaru et al. (1996) |
| Syphilis | Bark | Unspecified, oral | *E. abyssinica* | | |
| Syphilis | Flowers | Infusion, oral | *E. abyssinica* | Uganda | Kamusiime et al. 1996) |
| Antimalarial | Leaves/roots | Decoction/ infusion, oral | *E. secluxii* | Tanzania | Gassler et al. (1995) |

**Table 2.** Antibacterial test results for the crude extracts.

| Solvent | Conc. (mg/disc) | *S. aureus* | | *B. subtilis* | | *E. coli* | |
|---|---|---|---|---|---|---|---|
| | | Zone (mm) | MIC (mg/ml) | Zone (mm) | MIC (mg/ml) | Zone (mm) | MIC (mg/ml) |
| Hexane | 0.5 | 6 | ND | 6 | ND | 6 | ND |
| DCM | 0.5 | 10 | ND | 9 | ND | 7 | ND |
| EtOAC | 0.5 | 13 | ND | 12 | 0.125 | 11 | ND |
| MeOH | 0.5 | 15 | ND | 14 | 0.125 | 14 | ND |
| Gentamycin | 10 µg/disc | 17 | | 17 | | 17 | |

MIC = Minimum inhibitory concentration, ND = not done.

measured. This method was also used for pure compounds isolated which include glutinosalactone A, glutinosalactone B, lupinifolin, sitosterol and 3 β-stigimasterol. Gentamycin was used as a positive control. Two standard fungal strains namely; *Candida albicans* and *Aspergilus niger,* obtained from the Department of Plant and Microbiology of Kenyatta University were used. A standard antifungal drug, Nystatin, was used as positive control and DMSO as the negative control. 1 ml of each of the following extracts; hexane, dichloromethane, ethyl acetate and methanol were aseptically mixed with 15 ml of PDA. A five-day old fungal culture was then inoculated in an inverted position at the centre of the plate and then incubated at room temperature. This was done by streaking the fungal spores from the base plate to the new plate. The diameter of the zone of inhibition was measured in the third, fourth and fifth day. This method was also used for pure compounds.

## RESULTS AND DISCUSSION

The DCM, EtOAc and MeOH extracts were active against all the three micro-organisms. MeOH extract showed highest activity of 15 mm against *S. aureus*. EtOAc was also highly active with its highest activity being 13 mm against *S. aureus*. DCM extract had mild activities of 10, 9, and 7 mm against *S. aureus, B. subtillis* and *E. coli* respectively (Table 2). The extracts were also active against fungi used in the tests (Table 3). Methanol extract showed highest activity against the tested micro-organisms. Methanol is the most polar solvent hence many compounds extracted in methanol have promotional and synergist effect.

Phytochemical analysis of the crude extracts yielded five compounds. The structures of the compounds were elucidated by standard spectroscopic techniques that included UV, IR, 1H NMR, 13C NMR, COSY, NOESY, HSQC and HMBC and comparison with published data.

### Compound 1

White crystals; IR $\lambda_{max.}$(KBr) 3136 $cm^{-1}$ hydroxyl (-OH), 2937 $cm^{-1}$ methylene (-$CH_2$-), 1735 $cm^{-1}$ δ-lactonic carbonyl and 1637 $cm^{-1}$olefinic group (>C=CH-); $^1$H NMR ($CD_3OD$, 400 MHz) δ 6.00 (1H, *s*, H-12), δ 3.84 (1H, *d*, *J* = 9.56 Hz, H-30a), δ 3.76 (2H, *s*, H-30), δ3.68 (1H, *d*, *J* = 9.12 Hz, H-24a), δ3.41 (1H, *d*, *J* = 9.04 Hz, H-24b), δ 2.41 (2H, *d*, *J* = 13.2 Hz, H-16), 2.36 (1H, *d*, *J* = 13.2 Hz, H-15), δ 2.24 (1H, *d*, *J* = 8.56 Hz, H-9), δ 2.20 (2H, *s*, H-2a), δ 2.18 (2H, *s*, H-11a), δ 1.96 (3H, *m*, H-11a), δ 1.89 (3H, *s*, H-18), δ 1.83 (3H, *m*, H-22a), δ 1.78 (1H, *m*, H-2a), δ 1.70 (1H, *m*, H-11b), δ 1.58 (2H, *m*, H-21a), δ 1.57 (2H, *m*, H-7a), δ 1.45 (5H, *m*, H-16b), δ 1.45 (5H, *m*, 15b), δ 1.43 (5H, *m*, H-7b), δ 1.42 (5H, *m*, H-6a), δ 1.42 (5H, *m*, H-6b) δ 1.39 (1H, *m*, H-5), δ 1.37 (1H, *s*, H-29), δ 1.30 (2H, *m*, H-1), δ 1.18 (1H, *s*, H-27), δ 1.03 (1H, *s*, H-23), δ 0.91 (1H, *s*, H-25) and δ 0.81 (1H, *s*, H-26); $^{13}$C NMR ($CD_3OD$, 150 MHz) δ 181.1 (C-8), δ 88.1 (C-20), δ 73.9 (C-19), δ 71.4 (C-3), δ 66.2 (C-24), δ 63.6 (C-30),δ 51.1 (C-18), δ 50.8 (C-5), δ 49.6 (C-9), δ 43.9 (C-4), δ 43.0 (C-14), δ 41.8 (C-8), δ 41.1 (C-17), δ 38.4 (C-10), δ 34.6 (C-7), δ 33.8 (C-1), δ 26.7 (C-15), δ 26.5 (C-16), δ26.0 (C-2), δ 25.9 (C-11), δ 25.9 (C-9), δ 25.6 (C-21), δ 25.2 (C-22), δ 23.4 (C-27), δ 22.8 (C-24), δ 19.8 (C-6), δ

**Table 3.** Antifungal test results for extracts from *E. excels.*

| Solvent | Concentration (mg / disc) | Zones of Inhibition in mm | |
|---|---|---|---|
| | | ***A. niger*** | ***C. albicans*** |
| Hexane | 0.5 | 7 | 6 |
| DCM | 0.5 | 14 | 12 |
| EtOAC | 0.5 | 10 | 11 |
| MeOH | 0.5 | 15 | 13 |
| Nystatin | 10 µg / disc | 16 | 16 |
| DMSO | 6 | 6 | 6 |

Standard Nystatin (10 µg / disc) was used as a control for the experiment and diameter of the disc was 6 mm; MeOH extract had strongest activity of 15 mm against *A. niger* and 13 mm against *C. albicans.* EtOAC extract exhibited an activity of 10 mm against *A. niger* and 11 mm against *C. albicans.* DCM extract exhibited a strong activity of 14 mm, respectively on *A. niger* 12 mm on *C. albicans* while hexane extract exhibited no activity on both micro-organism. Positive control Nystatin had an inhibition zone of 16 mm.

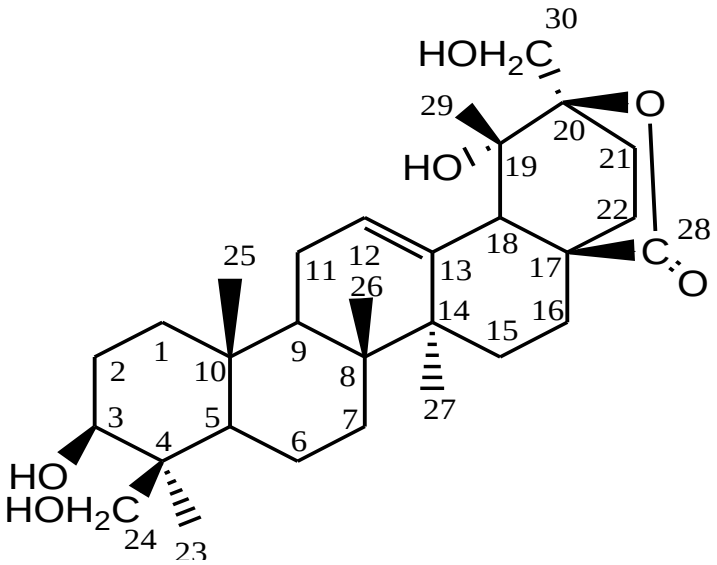


**Figure 1.** Compound 1: Gutinosalactone.

16.5 (C-26), δ 16.3 (C-25).

The isolated compound was white crystalline solid. It was visualized on TLC plate by spraying with anisaldehyde warming at 110°C and showed a characteristic purple colour confirming the presence of a terpenoid. The IR spectrum showed characteristic peaks at 3136 $cm^{-1}$ (broad) for hydroxyl (-OH), 2937 $cm^{-1}$ for methylene (-$CH_2$-), 1735 $cm^{-1}$ for δ-lactonic carbonyl and 1637 $cm^{-1}$ for olefinic group. The $^1$H NMR spectrum revealed a total of 20 proton signals resolved by analysis of COSY, NOESY, DEPT and HSQC spectra. The proton singlet resonating at δ 6.00 (1H, *s*, H-12) showed significant correlation from COSY spectrum with (H-11a) and NOE correlation with (H-29) from the NOESY spectrum. The $^{13}$C NMR spectrum showed 30 carbon signals, characteristic of a pentacyclic triterpenoid (Ahmad et al., 1996) as resolved by DEPT spectrum of which there were 8 quaternary carbons, three of which were highly deshielded oxygenated centers at δ 181.1 (C-28), δ 88.1 (C-20), δ 73.9 (C-19), four being points of attachment of methyl groups in the pentacyclic ring at δ 43.9 (C-4), δ 43.0 (C-14), δ 41.8 (C-8) and δ 38.4 (C-10), and one forming the lactonic carbonyl at δ 41.1 (C-17). There were 4 methine carbons identified at 71.4 (C-3), δ 51.1 (C-18), δ 49.6 (C-9), and δ 50.8 (C-5). The 11 methylene carbons were assigned to; δ 66.2 (C-24), δ 63.6 (C-30), δ 34.6 (C-7), δ 33.8 (C-1), δ 26.7 (C-15), δ 26.5 (C-16), δ 26.0 (C-2), δ 25.9 (C-11), δ 25.6 (C-21), δ 25.2 (C-22) and δ 19.8 (C-6).

The direct bonding of proton to carbon atoms were derived from the HSQC and HMBC spectra and the structure of compound 1 (Figure 1) was elucidated to be 3β, 19α, 20β, 24, 30-pentahydroxyurs 12-en-28-oic acid δ-lactone, $C_{30}H_{46}O_6$ commonly known as glutinosalactone

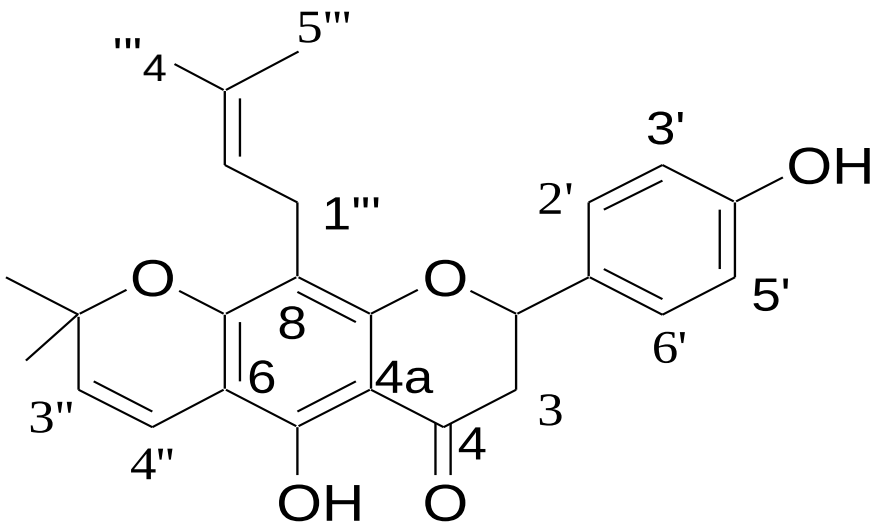


**Figure 2.** Compound 3: Lupinifolin.

A (Zhang et al., 2013). This is the first time extraction from this plant *E. excelsa.*

**Compound 2**

White crystals; IR $\lambda_{max.}$(KBr) 3137 $cm^{-1}$ hydroxyl (-OH), 2937 $cm^{-1}$ methylene (-$CH_2$-), 1735 $cm^{-1}$ δ-lactonic carbonyl and 1637 $cm^{-1}$ olefinic group (>C=CH-); $^1$H NMR ($CD_3OD$, 400 MHz) δ 6.00 (1H, *s*, H-12), δ 3.82 (1H, *d*, *J* = 9.56 Hz, H-30a), δ 3.76 (2H, *s*, H-3), δ 3.68 (1H, *d*, *J* = 9.12 Hz, H-24a), δ 3.41 (1H, *d*, *J* = 9.04 Hz, H-24b), δ 2.41 (2H, *d*, *J* = 13.2 Hz, H-16), 2.36 (1H, *d*, *J* = 13.2 Hz, H-15), δ 2.24 (1H, *d*, *J* = 8.56 Hz, H-9), δ 2.20 (2H, *s*, H-2a), δ 2.18 (2H, *s*, H-11a), δ 1.96 (3H, *m*, H-11a), δ 1.89 (3H, *s*, H-18), δ 1.83 (3H, *m*, H-22a), δ 1.78 (1H, *m*, H-2a), δ 1.70 (1H, *m*, H-11b), δ 1.58 (2H, *m*, H-21a), δ 1.57 (2H, *m*, H-7a), δ 1.45 (5H, *m*, H-16b), δ 1.45(5H, *m*, 15b), δ 1.43(5H, *m*, H-7b), δ 1.42 (5H, *m*, H-6a), δ 1.42 (5H, *m*, H-6b) δ 1.39 (1H, *m*, H-5), δ 1.37 (1H, *s*, H-29), δ 1.30 (2H, *m*, H-1), δ 1.18 (1H, *s*, H-27), δ 1.03 (1H, *s*, H-23), δ 0.91 (1H, *s*, H-25) and δ 0.81 (1H, *s*, H-26); $^{13}$C NMR ($CD_3OD$, 150 MHz ) δ 181.1 (C-8), δ 88.1 (C-20), δ 73.9 (C-19), 71.4 (C-3), δ 66.2 (C-24), δ 63.6 (C-30), δ 51.1 (C-18), δ 50.8 (C-5), δ 49.6 (C-9), δ 43.9 (C-4), δ 43.0 (C-14), δ 41.8 (C-8), δ 41.1 (C-17), δ 38.4 (C-10), δ 34.6 (C-7), δ 33.8 (C-1), δ 26.7 (C-15), δ 26.5 (C-16), δ 26.0 (C-2), δ 25.9 (C-11), δ 25.9 (C-9), δ 25.6 (C-21), δ 25.2 (C-22), δ 23.4 (C-27), δ 22.8 (C-24), δ 19.8 (C-6), δ 16.5 (C-26), δ 16.3 (C-25).

The structure of compound **2** was found to be similar to that of **1** with the OH in C-24 replaced by H and was elucidated as 3β,19α,20β,30-tetrahydoxyurs-12-en-28-oic acid lactone $C_{30}H_{46}O_5$ commonly known as glutinosalactone B.

**Compound 3**

Obtained as yellow crystals; mp. 116-119°C. $^1$H-NMR ($CDCl_3$, 200 MHz) 7.35 (*d*, J=8.8, H-2'/6'), 6.89 (*d*, J=8.6, H-3'/5'), 6.66 (*d*, J=10.0, H-4''), 5.52 (*d*, J=10.8, H-3''), 5.37 (*dd*, J=3.6, 12.6, H-2), 5.14 (*t*, J = 7.6, H-2'''), 3.22 (*d*, J=7.8, H-1'''), 3.05 (*dd*, J=12.6, 17.2, H-3ax), 2.82 (*dd*, J=3.2, 17.0, H-3eq), 1.65 (*s*, H-4'''), 1.65 (*s*, H-5'''); $^{13}$C-NMR ($CDCl_3$ 50 MHz) δ 196.4 (C-4), 160.0 (C-7), 159.4 (C-8a), 156.5 (C-5), 156.0 (C-4'), 131.1 (C-1'), 127.7 (C-2'/6'), 126.0 (C-3'''), 122.5 (C-2'''), 115.6 (C-4'''), 115.5 (C-3'/5'), 108.5 (C-8), 102.6 (C-4a), 102.6 (C-6), 80.1 (C-2''), 78.5 (C-2), 43.3 (C-3), 25.8 (C-4'''), 21.5 (C-1'''), 17.8 (C-5''') (Figure 2).

The compound was isolated from the DCM crude extract as yellow crystals; mp. 116-119°C. It was analyzed by $^1$H NMR spectrum as follows; the first signal was identified as a hydroxyl proton singlet stabilized by chelation with the carbonyl group and resonating at δ 12.24 (1H, *s*, H-1). The second signal integrating into two protons was identified as an aromatic proton doublet resonating at δ 7.35 (2H, *d*, *J* = 8.8Hz, H-2´, H-6´) which showed significant correlation from COSY spectrum with (H-3´, H-5´). The third signal integrating into two protons was aromatic proton doublet resonating at δ 6.89 (2H, *d*, *J* = 8.6 Hz, H-3´, H-5´). The fourth signal was a vinylic proton doublet resonating at δ 6.66 (1H, *d*, *J* = 10Hz, H-4´´). The fifth signal was a proton doublet δ 5.52 (1H, *dJ* = 10.8 Hz, H-3´´). The sixth signal was a proton double doublet δ5.37 (1H, *dd*, *J* = 3.6 Hz, *J* = 12.6 Hz, H-2). The seventh signal was a proton assigned as δ 5.14 (1H, *t*, *J* = 7.8 Hz, H-2´´´) which showed COSY correlation with (H-1´´´). The eighth signal was assigned as δ 3.22 (2H, *d,* *J* = 7.8 Hz, H-1´´´). The ninth signal was a double doublet assigned 3.05 (1H, *dd*, *J* = 12.6 Hz, *J* = 17.2 Hz, H-3ax).

The tenth signal was assigned as δ 2.82 (1H, *dd*, *J* = 3.2Hz, *J* = 17.0 Hz, H-3eq). The eleventh signal was identified as a proton singlet δ 1.65 (6H, *s*, H-4´´´/ H-5´´´). The twelfth signal was assigned as δ 1.45 (3H, *s*, H-$Me_2$) (Yenesew et al., 2009).

The $^{13}$C NMR spectrum showed 21 carbon signals. The hydroxylated methine carbon was identified as δ 78.5 (C-2) and a methylene carbon resonating at δ 43.3 (C-3). The highly deshielded quarternary carbon was identified as a carbonyl carbon at δ 196.4 (C-4). The quartenary aromatic carbons were identified as, δ 102.6 (C-4a), δ 156.5(C-5), δ 102.8 (C-6), δ 160.0 (C-7), δ 108.5 (C-8) and δ 159.5 (C-8a). The other aromatic carbon atoms were identified as δ 131.1 (C-1´), δ 127.7 (C-2´/6´) and δ 115.6 (C-3´/5´). The prenyl ring showed shifts at δ 80.0 (C-2´´), δ 126.0 (C-3´´), δ 115.6 (C-4´´). The hydroxylated olefinic group attached to benzene showed chemical shifts at δ 21.5 (C-1´´´), δ 122.5 (C-2´´´), δ 25.8 (C-4´´´) and δ 17.8 (C-5´´´). Most chemical shifts were in close agreement with those of lupinifolin (Yenesew et al., 2009).

**Compound 4**

White crystals in chloroform; mp. 131-133°C. The IR $\lambda_{max}$ (KBr) 3423 cm$^{-1}$; $^1$H-NMR ($CDCl_3$, 200 MHz) δ 5.20 (1H, t), 3.22 (1H, m), 1.06 (3H, s), 0.90 (3H, s), 0.85 (3H, s), 0.80 (9H, s); $^{13}$C-NMR (Chloroform 75 MHz) δ 139.6 (C-5), 124.4 (C-6), 79.1 (C-3), 59.1(C-17), 55.2 (C-14), 47.7 (C-9), 46.9 (C-24), 42.1 (C-4), 41.5 (C-13), 39.7 (C-12), 37.1 (C-1), 37.0 (C-10), 34.8 (C-20), 33.8 (C-22), 31.9 (C-8), 31.3 (C-2), 31.1 (C-7), 29.4 (C-25), 28.1 (C-16), 26.2 (C-23), 23.4 (C-28), 23.3 (C-19), 23.3 (C-21), 23.3 (C-15), 21.4 (C-11), 18.0 (C-26), 17.5 (C-27), 14.1 (C-18), 14.1 (C-29).

The compound was obtained from hexane/ DCM extract, with a melting point of 128 to 131°C. On the TLC plate the compound had an $R_f$ of 0.6 in hexane-DCM (1:1). When the plates were sprayed with p-anisaldehyde the spot turned purple suggesting that the compound was a triterpenoid (Dey and Harborne, 1991). The $^1$H-NMR spectrum displayed three regions namely; aliphatic, hydroxylated and allylic regions. The signals appearing as triplet at δ 5.20 suggested the presence of a double bond at a quaternary carbon atom. A multiplet centred at δ 3.22 was a characteristic of a proton germinal to a hydroxyl group at C-3 of triterpenoids. Six signals representing methyl groups were observed at δ 0.90, 0.96, 0.98, 1.00, 1.06 and 1.25 which are characteristics of a triterpenoid (Ahmad et al., 1996).

$^{13}$C-NMR spectra displayed signals of olefinic carbon atoms at δ 139.6 and 124.4 supporting the presence of a double bond at the end of a fused ring between C-5 and C-6 (Ahmad et al., 1996). The shifts at 14.1, 15.5, 16.8, 18.0, 23.4 and 18.4 were assigned to the methyl groups. The spectral data of compound 59 were in close agreement with those of 3β-sitosterol, whose structure is shown below (Alam et al., 1996).

**Compound 5**

White crystals soluble in chloroform, mp. 174-176°C; The IR $\lambda_{max}$ (KBr) 3423 cm$^{-1}$; $^1$H-NMR ($CDCl_3$, 200 MHz) δ 5.35 (1H, m), 5.15 (1H, dd), 5.05 (1H, dd), 3.51 (1H, m), 1.10 (6H, s), 1.05 (3H,s) 1.00 (3H, s), 0.90 (6H, s); $^{13}$C-NMR ($CDCl_3$, 75 MHz) δ 140.8 (C-5), 138.3 (C-22), 129.3 (C-23), 121.7 (C-6), 71.8 (C-3), 56.9 (C-17), 56.0 (C-14), 51.3 (C-24), 50.2 (C-9), 42.3 (C-4, 13), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.1 (C-20), 31.9 (C-26), 31.9 (C-25), 31.9 (C-2), 28.3 (C-16), 25.4 (C-28), 24.3 (C-15), 21.1(C-11, 21), 19.4 (C-19), 19.1 (C-27), 12.2 (C-29), 11.9 (C-18).

Compound 60 also obtained from hexane/ DCM extract, had a melting point of 173 to 175°C. On the TLC plate the compound had an $R_f$ of 0.6 in hexane-DCM (1:1). When the plates were sprayed with p-anisaldehyde the spot turned purple suggesting that the compound was a triterpenoid (Dey and Harborne, 1991). The $^1$H-NMR spectrum displayed several signals between 1.25 and 0.64 characteristic of a triterpenoid. The diagnostic chemical Shift values of the methyl protons for C-18 and C-19 appeared as singlets at δ 1.07 and 1.25 respectively. Four methyl groups appeared at δ 0.92, 0.93, 1.00 and 1.02 (Dey and Harborne, 1991). The multiplet at δ 3.51 suggested the presence of a proton attached to a hydroxylated carbon atom. A doublet at δ 5.35 suggested the presence of a double bond at a quaternary carbon atom. Two doublets of a doublet signal at δ 5.15 and 5.02 suggested the presence of a double bond on the side chain of a triterpenoid.

$^{13}$C-NMR spectra displayed 29 carbon atoms confirming compound **5** (Figure 4) was a modified triterpenoid. It showed signals at δ 140.8 and 121.7 confirming the presence of olefinic carbon atoms with more deshielded signals assignable to quaternary carbon at the bridge. The signal at δ 138.3 and 129.3 that lacked in the spectra of compound **4** (Figure 3) represented the olefinic carbons at the side chain. The peak at δ 71.8 was assigned to C-3 due to the presence of the hydroxyl group common to this class of compounds. Three signals at δ 36.1, 36.5 and 42.3 were associated with the three quaternary carbon atoms. The spectral data compared closely to that of 3β-stigmasterol, whose proposed structure is shown below (Alam et al., 1996). This compound is being reported from *E. excelsa* for the first time. Glutinosalactone A (**1)** and glutinosalactone B (**2)** exhibited high activity against all the three micro-organisms in which glutinosalactone A (**1**) had activities of 11, 11 and 10 mm against *S. aureus*, *B. subtillis*, and *E. coli*, respectively while glutinosalactone B 2 had activities of 10, 11 and 10 mm against *S. aureus*, *B. subtillis* and *E. coli,* respectively. Lupinifolin (**3**) had mild

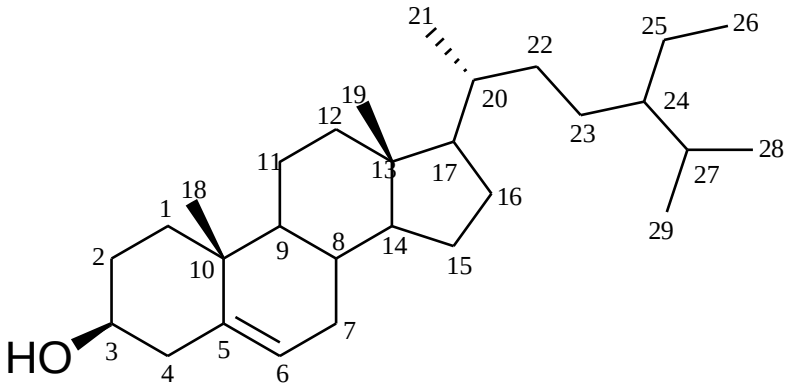


**Figure 3.** Compound 4: Sitosterol.

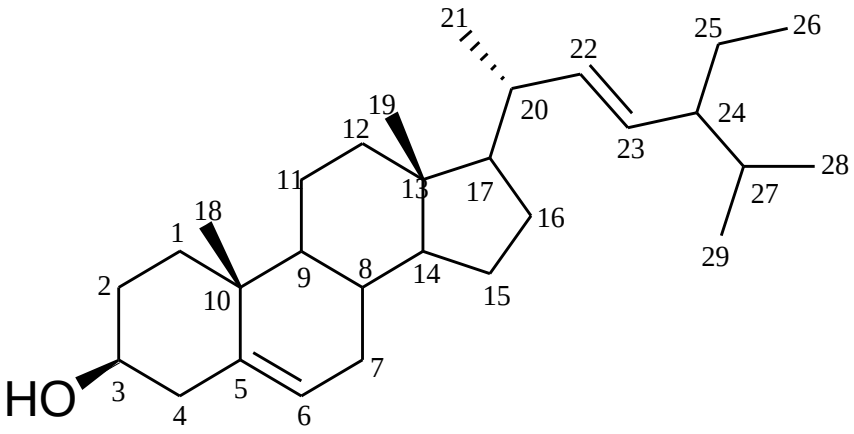


**Figure 4.** Compound 5: Stigimasterol.

**Table 4.** Antibacterial and antifungal activities of the isolated compounds from the stem bark.

| Compound | *S. aureus* | *B. subtillis* | *E. coli* | *A. niger* | *C. albicans* |
|---|---|---|---|---|---|
| Glutinosalactone A **1** | **11** | **11** | **10** | 15 | 11 |
| Glutinosalactone B **2** | **10** | **11** | **10** | 10 | 11 |
| Lupinifolin **3** | **8** | **8** | **8** | 9 | 9 |
| 3β- sitosterol **4** | **7** | **6** | **6** | 8 | 6 |
| 3β- stigimasterol **5** | **7** | **6** | **6** | 8 | 6 |
| Gentamycin (+ve control) | **17** | **17** | **17** | 16 | 16 |
| Nystatin (+ve control) | **-** | **-** | **-** | 16 | 16 |

activity of 8 mm against all the three micro-organisms. 3β-sitosterol (**4**), 3β- stigimasterol (**5**) had mild activity on *S. aureus* only. Glutinosalactone A (**1**) had the highest activity with inhibition zones of 15 mm against *A. niger* and 11 mm against *C. albicans* while glutinosalactone B (**2**) had activity of 10 mm against *A. niger* and 11 mm against *C. albicans*. Lupinifolin **(3)** was moderately active with inhibition zone of 9 mm against the two micro-organisms. 3β sitosterol and 3β stigimasterol were both inactive in C. albicans but had mild activities of 8 mm respectively against *A. niger* (Table 4).

*E. excelsa* has a wide range of phytochemical

components responsible for its biological activity. Amongst the phytochemicals identified are flavonoids and terpenoids. From these results it is evident that the crude extracts had substantial activity against tested micro-organism but the activities of the isolated compounds were much lower. This is attributed to the synergistic, antagonistic and promotional effects resulting from the various compounds that were present in the crude extracts (Gathirwa et al., 2008). At the same time the methanol extract showed highest activity because it is the most polar solvent hence during extraction several compounds in this extract brings about synergism.

## Conclusions

The reported activities of crude extracts and isolated compounds justify the usage of *E. excelsa* as an anti-dote for snake bites and in treatment of arthritis by the communities in Western Kenya. The plant extracts have diverse compounds, flavonoids and terpenoids included. Methanol, ethyl acetate and dichloromethane extracts were active against some bacteria and fungi. Methanol extract had strongest activity of 15 mm against *S. aureus*, 15 mm on *A. niger* and 13 mm on *C. albicans.* 14 and 12 mm were recorded against *A. niger* and *C. albicans* respectively in the dichloromethane.

Phytochemical characterization of compounds isolated from the stem bark of the plant resulted in the isolation of four triterpenoids and one flavonoid. Methanol extracts contained, triterpenoids; glutinosalactone A (**1**) and glutinosalactone B (**2)** were isolated. From hexane-DCM extract, Lupinifolin (**3**), sitosterol (**4**) and 3β-stigimasterol (**5**) were obtained. Glutinosalactone A (**1**) had highest activity of 11 mm against *S. aureus* and *B. subtillis* while glutinosalactone B (**2)** had high activity of 11 mm against *B. subtillis*. This justifies the greatest activity of the MeOH crude extract. Lupinifolin **(3)** had mild activity of 8 mm against all the three micro-organisms. Glutinosalactone A (**1**) exhibited an inhibition zone of 15 mm against *A. niger* and 11 mm against *C. albicans* while glutinosalactone B (**2**) had an inhibition zone of 10 mm against *A. niger* and 11 mm against *C. albicans.* Flavonoid **(3)** had an activity of 11 and 9 mm against *A. niger* and *C. albicans*, respectively. Sitosterol (**4**) and 3β-stigimasterol (**5**) had mild activity against *A. niger* and were inactive against *C. albicans*.

It is evident that crude extracts contains many compounds and this accounts for the high activity of the crude compared to the isolated pure compounds. This many compounds bring about promotional and synergist effects (Gathirwa et al., 2008).

## RECOMMENDATIONS

Further studies should be done on other parts of the plant such as the leaves, roots and the seeds to isolate more bioactive compounds

## Conflict of Interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors are greatly indebted to VicRes for financial support of this research.

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