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Evaluation of Some Phytochemical Tests for Gumbail (*Cordia africana Lam.*) and its Uses in Termite Management

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A B S T R A C T

Sudan has great divergence in natural resources; forests for example play a crucial role in providing wide range of products. Gumbail tree is of wide distribution in it. The aim of this work was to evaluate some phytochemical tests for Gumbail (*Cordia africana*) and its uses in termite management. The phytochemical evaluation involves the separation of the active ingredients from leaves-ethyl acetate extract to control termites. The analysis of chromatography showed ten bands. Dursban 48EC was also used as standard in the termite management and it's the average termite's damage was (13.22% weight loss) comparing to the above ten bands. The results showed that, the average termite's damage of band number (1) was (20.56% weight loss) and it's more efficient than the other bands. The active ingredients were detected in intact and seedling of plant samples from Zalingei and Diling, by thin layer chromatography. Finding novel termite-active compound in Gumbail was recommended to use as wood-paints or pesticide to management termite.

Introduction

Cordia africana is deciduous tree up to 30 m tall, characterized by very dense foliage consisting of large, dark green, cordial leaves (Bekele - Tesemma *et al*., 1993). It is a large shady tree spreading for 10 m. The local name in Sudan is "gumbail" (Bein, 1996). It's also wide spread in tropical Africa. In Sudan, the tree is confined to the areas of Ad-Damazin, Darfor and Kordofan (Drummond, 1981). *Cordia africana* is a species of flowering tree that belong to Boraginaceae family (Nadja*et al*., 2002). This multipurpose tree is valued for food, fuel, and relatively termite resistant and timber; it is used for high quality furniture (Bekele – Tesemma *et al*., 1993).

Secondary metabolites are known to play a major role in the adaptation of plants to their environment (Bourgaud *et al*., 2001). Recently, plants possessing insecticidal substances have generated extraordinary interest as potential sources of natural insect control agents (Murray, 2006).Efficacy of various plants and their extracts have been examined in *invitro* and field conditions but still there is a need to develop suitable efficacious botanical based formulations to tackle the losses caused by termites (Dokurugu *et al*., 2012). The productivity of forests and agricultural crops are generally affected by frequent outbreak of pests which causes lot of economic loss. One among the common pests that affect the crop plants are termites (Sahay *et al*., 2014).

Termites belong to order Isoptera. They live in huge mounds, in colonies and feed on cellulose material and on almost anything which contains carbohydrate. They are known to cause tremendous loss to finish and unfinished wooden structures in buildings. Termites infest many commercially important plants (Sahay *et al*., 2014). The damage caused by termites alone is reported to be more than the combined annual destruction caused by fires, tornadoes and earthquakes in monetary terms (Isman, 2006).

Although different parts of *Cordia africana* (Gumbail) are thought to be either toxic or repellent to termites, no specific work has been done to extract active compounds for the control of termites. In Sudan related to this is the fact that, insecticides are expensive and not locally available. Botanical insecticides for example, can be used as alternative to synthetic insecticides. The present study was aimed to evaluate the efficiency of active compounds extracted from locally occurring *Cordia Africana* plants with chromatography methods for management termite.

Materials and Methods

Extraction procedure

Fresh and healthy sample of leaves from naturally growing plants of *C. africana* was (randomly) collected from Zalignei area to be shade dried and powdered prior to extraction.

The collected seeds (originally from Zalingei and Diling area) were sown inpots and left to grow in the green house. Samples of *C. africana* leaves from seedlings were collected after 4-6 months of growth, to be shade dried then ground prior to extraction. The pulverized samples (each 40 g) were placed in a thimble and extracted using ethyl acetate in a soxhlet apparatus. The extraction was carried out until a clear solvent was observed. Extracts were further concentrated in a rotary evaporator at temperature adjusted according to the solvent used (Houtman *et al*., 2007).

The phytochemical screening ofethyl acetate leaves extract of intact plant from Zalignei first was separated with column chromatography (CC) and the separated compounds were further analyzed by means of thin layer chromatography (TLC), then assayed for their efficiency as anti-termite or termite repellency using the grave yard method. Second was a gas chromatography-mass spectroscopy (GC-MS) technique. Ethyl acetate leaves extract of *C. africana* from Zalingei and Diling and other from their seedlings were subjected to TLC profiling.

Column chromatography

Two grams from the dried ethyl acetate leaves extract of intact plant from Zalignei were suspended in 5 ml ethyl acetate, and subjected to column chromatography, using

a clean, dry column aligned in a vertical position. The column is 37x2 cm filled with silica gel (mesh size: 60-120) to a level of 8 cm from the top and soaked with petroleum ether for at least 12 hours. The immobilized extract was added gently to the free volume at the top of the column via a funnel. After bedding down through the gel material, fractionation was conducted by successive sequential application of solvent gradients, according to polarity, which prepared from a series of solvent system, petroleum ether, chloroform and ethyl acetate (each system 100 ml). The flow rate of the mobile phase was controlled by adjusting the opening of the outlet valve (1.3 ml/min). The fractions (20 ml each) were collected in separate test tubes, and the solvent was carefully removed by rotary evaporation. Column development was repeated until produce enough amount. Dried fractions were suspended in ethyl acetate (2 ml) and diluted when necessary prior to analytical thin layer chromatography onglass micro-platesand biomass assay (Reid and Sarker, 1998).

Thin layer chromatography (TLC)

Preparation of TLC plates

Separation of individual compounds in the fractions which obtained from column chromatography was carried out using two types of TLC analysis; these are commercially readymade pre-coated TLC plate of size 20x20 cm^2, 0.2mm thickness and the glass micro-plates prepared in the laboratory. The TLC plates were made by mixing the adsorbent (silica gel), containing small amount of calcium sulphate (gypsum) as an inert binder, with twice the volume of distilled water (Van Sumere *et al*., 1965). The mixture was spread as slurry on previously cleaned glass micro-plates using a TLC spreader of 0.5 mm thick layer. The plates were set to be air dried at room temperature for 30 minutes and activated at 110˚C in an oven for 30 minutes.

Fractionation of the extract

Samples of individual compounds were drawn with capillary tubes (glass micro-plates) or micro syringe (pre-coated plate, 30 µl) and spotted on stationary phase in a line at about 2 cm from the bottom. The TLC plates were developed in saturation chromatography tank using mobile phase solvent system composed of petroleum ether: chloroform: ethyl acetate in a ratio of 2:2:1, respectively. Prior to development, the solvent was poured into chromatography tank, covered and allowed to saturate at room temperature (28˚C±2˚C) for 30 minutes. The plate loaded with the samples was then carefully placed into the chromatography tank. At the end of the chromatography development, the plate was removed out and left to dry at room temperature (Kotze and Eloff, 2002). The separated spots were visualized under daylight (visible light) and under Ultra-Violet (UV) light (UV_{254} nm and UV_{366} nm), to detect the phytochemicals (Stahil, 1969). Distances between the spots and the end of the mobile phase were measured, and the retention factor (R_f) values were calculated and recorded (Schlitt and Geiss, 1972)using the following:

$$R_f \text{ Value} = \frac{\text{Distance moved by the compound}}{\text{distance moved by the solvent front}}$$

Assay of anti-termite activity

The components of acetyl acetate leaves extract with column chromatography were assayed for anti-termite activity. An amount of 0.1g of each component was dissolved in 15 ml ethyl acetate. To standardize the concentration for all parameters the equation "$C_1\ V_1 = C_2\ V_2$" was used to obtain the

following concentrations: C_1= 6.6 ppm,C_2 = 4.5 ppm, C_3 = 3.0 ppm, C_4 = 2.0 ppm, C_5 = 1.0 ppm. The obtained concentrations of the different fractions were separately spread on single layer cellulose pads with dimensions of 0.2 cm X 10 cm X 10 cm till moistened. Pads moistened with Dursban 48ECwas used for comparison. Untreated pads were used a control. The moistened pads were left to evaporate till drying and then labeled. Three replicas were used for each treatment. All the labeled pads were weighed before being buried at the testing site using the grave yard method (ASTM, 1989). The test was carried out at the experimental farm of the Gezira University in a site well known with termite infestation. The test pads were distributed randomly in a completely randomized design (C R D), and designated with vertical labels (Gary, 1979). The grave yard test lasted for 5 days. The samples of experiment were harvested, assessed visually, cleaned and weighed, to find the loss in weight due to the action of termites.

Statistical analysis

Data of experiment for assay of anti-termite activity of extract were statistically analyzed using analysis of variance (ANOVA) M. STAT. Program, and presented as average, standard error. Means were obtained using Duncan's multiple range test (DMRT) (Duncan, 1955). Data was transformed to $\sqrt{x+1}$ when necessary.

Gas Chromatography Mass Spectrometry (GC-MS)

Standard commercial anti-termite (Dursban 48EC) and the number one fraction of the TLC were separately dissolved in ethyl acetate for the purpose of developing the chromatography. One µl of each fraction was drawn for GC-MS analysis, which was carried out as described by Zhang and Zuo (2004). A Varian GC-MS (QP-2010 SHIMADZO-JAPAN) equipped with a split/splitless programmable temperature injector and capillary column (30 m length, 0.25µm diameter) was employed for the study, the GC oven temperature was programmed as follows: initial 3 minutes at 50°C, 10°C/min. to 200°C, then 8°C/min. to 280°C and held for 5 minutes. The total run time was 33 minutes. The ion-trap mass spectrometer was operated and in full scan mode from m/z 40-650 for qualitative analysis. The peak area and retention time were recorded (Carmen *et al*., 2000).

Results and Discussion

Column chromatography

In order to isolate the bioactive compound from the crude extract it was further fractionated using column chromatography. Then the column was eluted with solvent gradients according to polarity to provide 93 fractions. Isolation of active compounds from ethyl acetate leaves extract of *C. africana* using column chromatography by preparative TLC resulted in 10 purified compounds. The compounds 1 and 2 were collected from the column fractions 2 -24. Compounds 1, 2 and 3 were collected from the column fractions 25 - 40. The compound 4 was isolated from the column fractions 41 - 45. The compounds 2, 3, 4 and 5 were collected from the column fractions 46 - 49. The compounds 4, 5, and 6 were collected from column fractions 50 - 53. The compounds 6, 7 and 8 were collected from the column fractions 54 - 56. The compounds 7 and 8 were collected from the column fractions 57 - 59. Compound 9 was isolated from the column fractions 60 - 70. Compound 10 was isolated from the column fractions 71-90. Repeated chromatographic separation of ethyl acetate extracts of leaf of *C. africana* provided a total of ten

compounds, their retention factor (R_f value) and yield showed in (Table 1). Results were clarified variation in yield among the compounds, where the highest yield (17.5%) was observed in compound 3 and lower yield (2.25%) was that of compound 6.The fractionation and qualitative analysis would be of great value to determine the contents of the extractive (Lijun and Curtis, 2006). Fresh or dried plant material can be used as a source for secondary plant components. However, most scientists working on the chemistry of secondary plant components have tended to use dried plant material for several reasons (Baris *et al*., 2006).

Profiling the compounds with TLC

Fractions of ethyl acetate leaves extract from column chromatography were subjected to TLC screening to the 10 compounds fractionated by column chromatography. The result showed that when plate was visualized on day light, there was development of 10 major bands with different colors and R_f values. The colors of the bands range from green and yellow to grey and brown. The range of colors was indicative of presence of different compounds with different polarities. All the ten compounds were present when the plates were visualized under UV_{254} and UV_{366} and there were no difference in the number of bands and their R_f values. The results of elution with TLC confirm the presence of secondary metabolites in ethyl acetate leaves extractof *C. africana*.

When ethyl acetate extract from Leaves of*C. africana* plants collected directly from their native areas (Zalingei and Diling) in addition ethyl acetate extract from leaves of plants of similar origins but grown on the green house, were subjected to TLC profiling, there was a difference in profile number 10 with green color of plants native from Zalingei (Z-0) which did not appear in the other profiles. Meanwhile, the compounds of samples Zalingei plant grown in green house, native plant from Diling and Diling plant grown in green house (Z-1, D-0 and D-1) were more or less similar in their number and distance of the bands of the profile. Colors of the bands in all profiles were the same except for the compound number 8 of the profile D-1 which appeared with green color, while it has yellow color in all other three profiles as visualized with daylight. This different in color was an evidence for the difference in the chemical constituent of the compounds. These results indicate that the different species occurring in different locality may have the same metabolites as shown in Figure (1).

Further anti-termite assay

The ten compounds were also assayed in different concentrations to control termite damage. The results showed that all rates of concentration of compounds and standard (Dursban 48EC) significantly reduced the damage caused by termites compared to the untreated control. Significant differences were observed between compounds in the control of termite attack. Also results revealed that, Dursban 48EC was (mean of 0%weight loss) at 2 ppm comparing to the ten compounds, while compound(1) was more efficient(mean of 3%weight loss) at same concentration than the other compounds, Enhanced results were obtained by compounds 1, 10, 7 where the average termite's damage were 20.56%, 27.56%, 30.89%, respectively, that means they have repelling activity against termites; however, their efficiencies were less than Dursban 48ECwas 13.22%. In general the poorer repellent effect was observed with compound 6 where the average damage of concentrations reached about 79.22% (Table 2).

Table.1 The concentration percentage and Rf values of compounds from ethyl acetate leaves extract of *C. africana*

Compound	Yield (%)	R_f
1	5.14	0.91
2	10.43	0.89
3	17.50	0.80
4	10.27	0.66
5	3.11	0.54
6	2.25	0.44
7	6.26	0.26
8	5.86	0.20
9	6.52	0.11
10	2.83	0.08

Table.2 Average termite damage on cellulose pads treated with different concentrations of compound fractions extracted from leaves of *C. africana*

Compound	Average termite damage (%)
1	20.56 ^{g}
2	57.89 ^{c}
3	56.89 ^{c}
4	54.83 ^{d}
5	63.22 ^{b}
6	79.22 ^{a}
7	30.89 ^{e}
8	63.61 ^{b}
9	58.44 ^{c}
10	27.56 ^{f}
Standard	13.22 ^{h}
SE±	0.615
C.V.%	60.39

*Means in each column followed by the same letter (s) are not significantly different at $P \leq 0.05$; according to Duncan's multiple Range Test.

* SE ±: Standard error.

* C.V. %: Coefficient variance.

Fig.1 TLC profiles under day light of compounds extracted from *C. africana* leaves
Z-0 = native plant from Zalingei, Z-1=Zalingei plant grown in green house
D-0= native plant from Diling, D-1= Diling plant grown in green house

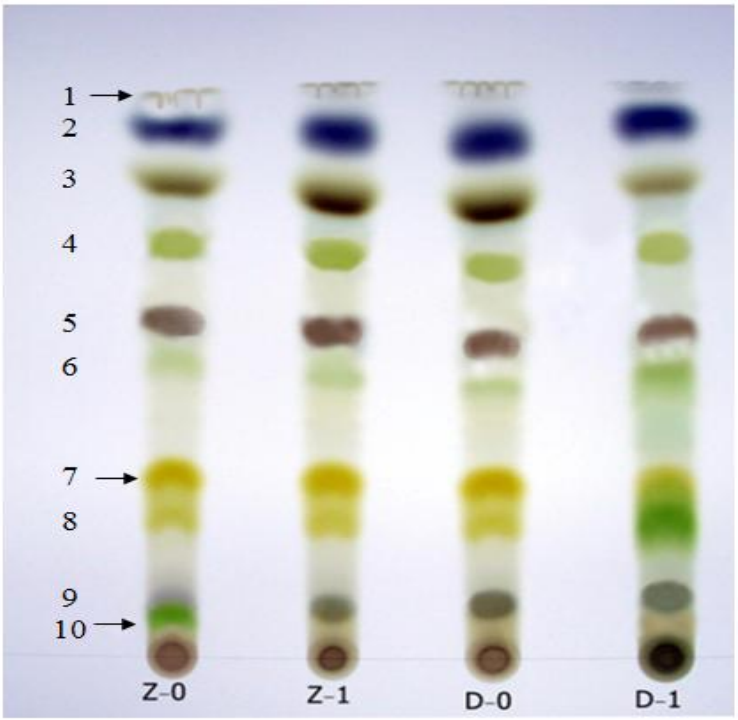
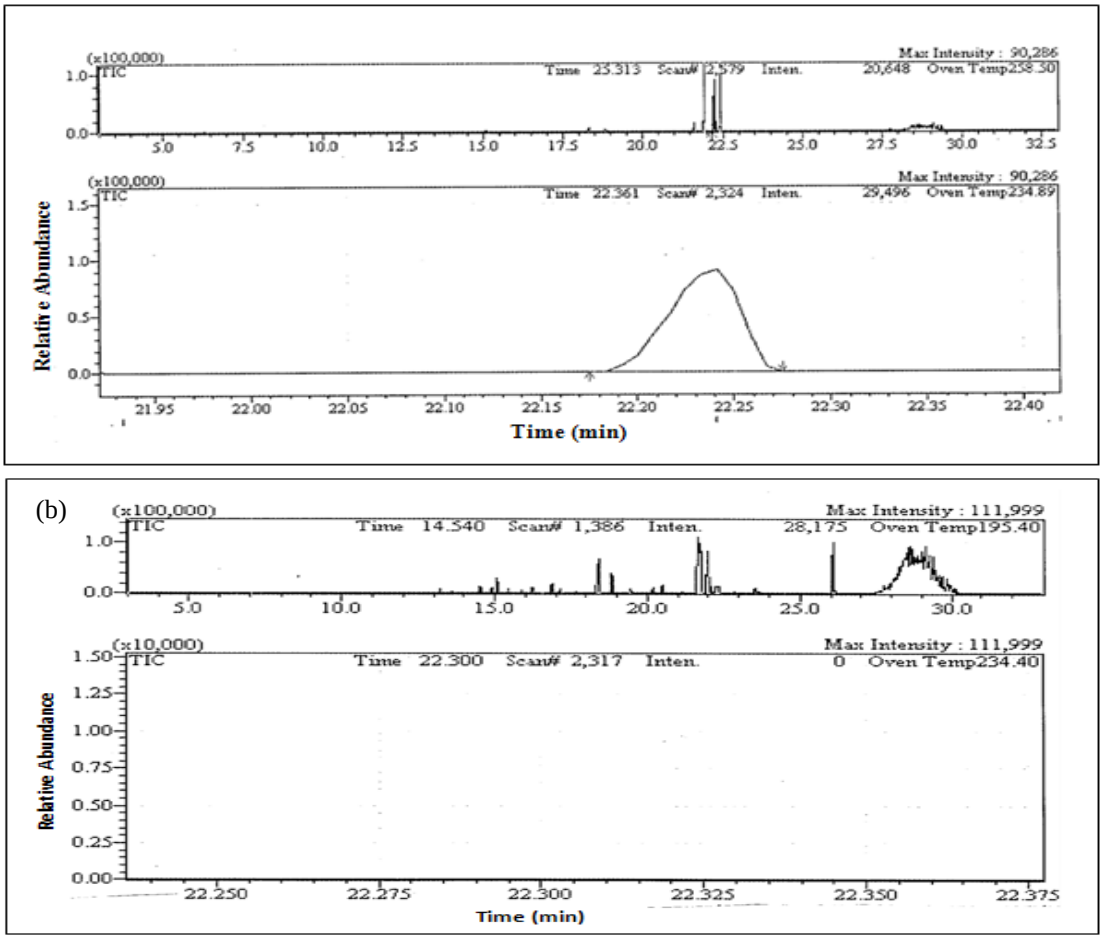


Fig.2 Effect of compounds extract with ethyl acetate from leaves of *C. africana* at different concentrations a) Treated cellulose pads before being burying in soil
b) Termite damage on treated cellulose pads

Fig.3 Chromatogram of 1 µl working (a) Dursban 48EC standard (b) compound one



In the majority of the compounds it was observed that, the tendency of increasing concentrations resulting in a decrease infestation (Figure 2). Evidently, results obtained showed repellent effect of *C. africana* on termite insects. Generally, compounds 1, 10, 7 have been indicated to possess strong repellant activity towards termite.

In fact, all the termiticides residues have a minimum threshold level to either kill or repel invading termites (Gold *et al*., 1996). Betty *et al*., (2001) reported that essential oils of cedar wood and cassia leaves had a good repellent effects, the repelling period was 3-6 days at the concentrations 10, 25and50µg /cm^2. Although a number of publications have focused on the isolation and identification of bioactive compounds, it is important to keep in mind that a single compound may not be responsible for the observed activity but rather a combination of compounds interacting in an additive or synergistic manner.

Identification for possible compound by GC-MS

The most potent compound (1) was subjected to Gas chromatography-Mass spectrometry (GC-MS) in comparison with the standard Dursban 48ECfor identification the possible active compound. The chromatograms results of the GC-MS analyses are shown in (Figure 3). In the

linear regression analysis of Dursban 48EC, a peak appeared at specific retention time (22.23 minutes) (Figure3 a). When the same concentration (1 µl) of compound (1) was injected in the GC-MS, no peak appeared at the same retention time (Figure 3 b). This result indicated that, Dursban 48EC was absent in our sample. Therefore, compound (1) was considered a promising novel natural product, which has highest repellant activity to termite. Further studies were attempted to reveal the nature of active ingredients by using chemical methods of chromatography and spectroscopy. GC-MS is a useful tool for quantitative and qualitative analysis of a wide range of relatively volatile compounds, and the technique has been widely applied in medical, biological, and food research (Sheille *et al*., 2002). However, GC-MS analysis is still the most widely used method for routine analysis, and care must be taken to optimize the chromatographic conditions in order to obtain the most accurate results.

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