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Potential antitermite compounds from *Juniperus procera* extracts

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Abstract

Thin layer chromatography (TLC) analysis revealed that destructive distillation of *Juniperus procera* tree gave ten major components, whereas *Croton megalocarpus* tree yielded five components. This was confirmed by gas chromatography (GC). The components were isolated by column chromatography and analysed using infrared, ultra-violet, visible and mass spectroscopy (MS) techniques. The whole extract was about 30.3% of the starting material (sawdust) and consisted of 77.5% water and 22.5% oily reddish-brown layer. The extracts had alcoholic and phenolic compounds together with acids. Cedrol, a tertiary tricyclic alcohol, was found to be in the greatest proportion in the oily layer. IR spectra with a peak beyond 3000 cm^{-1}, UV-VIS absorption maxima at 230 nm and mass spectra with *m/e* 204 suggested the presence of cedrene in the extract.

Keywords: Termite control; Natural pesticide

1. Introduction

The present research project is a spinoff of research work aimed at obtaining anti-termite compounds by destructive distillation of wood sawdust (Wilkins, 1990). Wood is composed of about 50% carbon, 6% hydrogen and 44% oxygen. Moreover, a large number of organic compounds, which can be extracted with organic solvents exists (Wise, 1952; Herman, 1970). It was in search of anti-termite formulation (Harris and East, 1943; Harris, 1971; Kari, 1992; Poswel and Akpa, 1993) that we directed our investigations to one of the indigenous local trees, *Juniperus procera* (African pencil cedar). The first phase of this work involved destructive (dry) distillation extraction of *J. procera* followed by characterization using column chromatography, thin layer chromatography (TLC), gas chromatography/mass spectroscopy (GC/MS). Thus GC/MS (De Mayo and Reed, 1956; Ryhage and Stenhagen, 1960; Budzikiewicz and Djerassi, 1962; Shriner et al., 1980) was used to verify the active ingredients found from extracts obtained by destructive distillation of *J. procera* and *Croton megalocarpus*.

2. Experimental

2.1. Chemicals

Dichloromethane, methanol, hexane and petroleum ether were bought from Kobian. Anhydrous sodium sulphate, activated charcoal, carbon tetrachloride and bromine were BDH products. All other chemicals were analytical reagent grade.

2.2. Apparatus and procedures

A Hewlett-Packard, VG 12-250 quadrupole GC/Mass Spec model equipped with a data system, was used

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to obtain chromatograms and mass spectra. Perkin Elmer 8500 GC was also used. UV-VIS spectra were acquired using Pye Unicam SP8-150 UV-VIS spectrometer. Vibrational frequencies were obtained using Pye Unicam SP 300.

TLC was performed on either commercially precoated or freshly prepared silical plates. A portion of *J. procera* was cut into pieces, dried and chopped into small pieces, which were dried further for four weeks. The pieces were further reduced in size and finally ground into finely divided sawdust. The dry sawdust (134.8 g) was gradually heated in one litre round bottom flask (pyrex) connected to a condenser and receiver. The distillate was collected over ice in sample vials, stoppered, weighed and stored in refrigerator. The extract layers were partitioned using a 25 ml separatory funnel.

Solvent mixture (300 ml) of *n*-hexane-dichloromethane (1:2) was used to extract 100 g of sawdust in a soxhlet extractor apparatus at 70°C.

A clean glass column (2.5 cm diameter and 1 m length), containing glass wool and acid-washed sand at the bottom, was packed with silica gel. The sample was introduced into the column followed by addition of eluting agent (200 ml hexane). This was followed by addition of 200 ml of 20% dichloromethane in hexane. On the average, the rate of collection was 2 ml per minute.

3. Results and discussion

3.1. TLC analyses

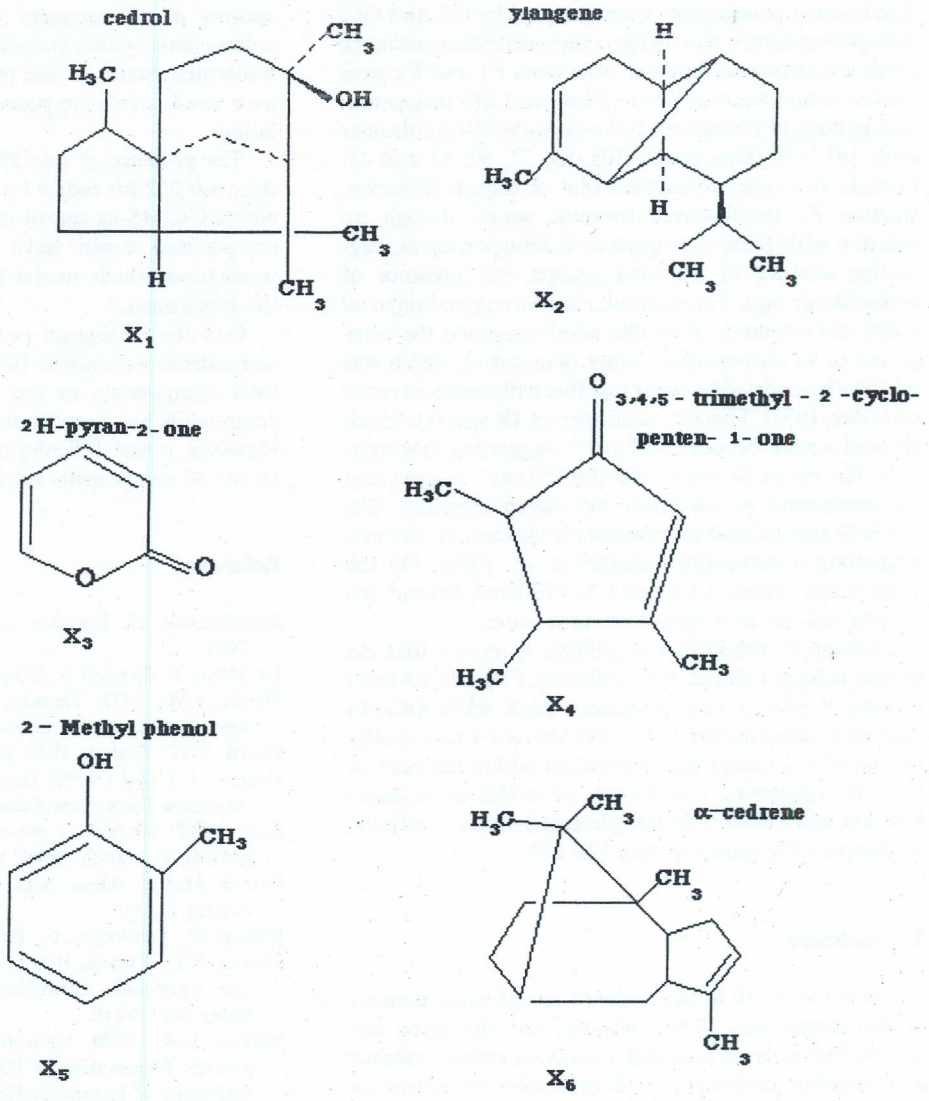
The work was done to find compounds with potential antitermite properties. Following dry distillation of sawdust of *J. procera* (African pencil cedar tree), the crude extract was 30.34% of the starting material. Partitioning and fractional distillation indicated that 77.5% (v/v) of the extract was water, whereas 22.5% (v/v) consisted of yellow and reddish-brown oily layers which were soluble in dichloromethane.

TLC analyses of *J. procera* crude extract indicated presence of ten distinct components with retardation factors (RF) of 0.93, 0.86, 0.84, 0.81, 0.71, 0.66, 0.64, 0.57, 0.31 and 0.20. On the other hand, soxhlet extracted *J. procera* sawdust gave seven components with RFs of 0.91, 0.83, 0.64, 0.54, 0.25, 0.16, and 0.11, suggesting that soxhlet extraction yielded fewer components than the dry distillation method. For comparison, sawdust of *C. megalocarpus* tree was subjected to destructive distillation extraction. The extracts exhibited TLC RF values: 0.72, 0.58, 0.50, 0.47, 0.33, 0.24 and 0.15, suggesting that *C. megarocarpus* had fewer components (7) than *J. Procera*.

3.2. Solvent extraction and GC and GC/MS analyses

The oily layer extract, which floated on top of the aqueous layer in a separatory funnel, was extracted with dichloromethane (system 1). Two layers were produced, the dichloromethane layer and a layer containing the rest of the extract. The latter layer was extracted with hexane (system 2). The aqueous layer of the crude extract was similary extracted, which gave two layers, the hexane portion (system 3) and the other layer containing water. The water phase was extracted with dichloromethane (system 4). Systems 1 to 4 were analysed by GC and GC/MS spectrometers. The Gas chromatogram of system 1 gave a major peak with a scan number 727. Other minor peaks appeared as impurities. The mass spectra of the peak at scan number 727 showed the largest mass ion peak at a *m/e* value of 204 and a base peak at *m/e* 119, coupled with an equally large peak of mass 93. This fragmentation corresponds to cedrol (X1) or ylangene, X2, (see Scheme 1) with molecular formulae $C_{15}H_{26}O$ (FW 222) and $C_{15}H_{24}$ (FW 204), respectively. For cedrol, the peak at *m/e* 204 would be as a result of a loss of a water molecule from the molecular ion of mass 222. In this case, the ion with *m/e* 204 would not be the molecular ion. A further loss of C_6H_{13} fragment gives the peak at mass 119. A further loss of C_2H_2 would give the peak at *m/e* 93.

The hexane-extracted oily portion (system 2) contained the same components as system 1, but in negligible quantities, suggesting that most of the components in the oily layer were insoluble in hexane. The chromatogram of system 3 exhibited several peaks. There were at least twelve components, though the most prominent peaks were eight. The major peak had a scan number of 573 (20.3 min), while other peaks had scan numbers 425, 447, 573, 625, 705, 999, 1125 and 1192. The mass spectra of the peak at scan number 447 gave a base peak of *m/e* 39 and a peak arising from molecular ion with *m/e* 82. The fragment mass 39 was due to $C_3H_3^+$. The molecular ion (*m/e* 82) loses CH_3 group to yield the peak of mass 67, suggesting presence of 3-methylfuran, C_5H_6O (FW 82). The peak of scan number 573 had a mass spectrum with major fragments of masses 39, 67, and 96 plus other smaller peaks. *M/e* of 96 was the molecular ion, which lost C_2H_5 to yield *m/e* 67. This fragments further to give the peak of mass 39. The most likely compound was 2H-pyran-2-one, $C_5H_4O_2$ (X3), FW 96. Another component with significant peaks was the one with a scan number 999. The MS spectra gave a parent ion of *m/e* 124 and a base peak *m/e* 109. Other fragments had masses of 81, 65, 53, 39,... A loss of CH_3 from the molecular ion of *m/e* 124 gives the base peak of *m/e* 109. Fragmentation of C_2H_3O from the molecular ion yields the peak at *m/e* 81. The likely structure corresponding to this spectra is that of the compound 3,4,5-trimethyl-2-cyclopenten-1-one (X4). The peak with scan



Scheme 1.

number 1125 gave a mass spectra with a molecular ion *m/e* 108 and a base peak of mass 94. Other fragments gave masses of 79, 77, 62, 50 and 39, suggesting the most likely compound to be 2-methylphenol (X5). The CH_3fragments from methylphenol, yielding *m/e* 93. Loss of OH give a peak at *m/e* 76. The phenyl group ($C_6H_5^+$) gives rise to peak of *m/e* 77.

The aqueous dichloromethane extract portion (system 4) contained essentially the same compounds as the hexane-extracted portion (system 3), though the peaks obtained were much smaller than those of system 3.

Overall, the compounds detected by GC/MS were cedrol ($C_{15}H_{26}O$), 3-methylfuran(C_5H_6O), 2-H-pyran-2-one ($C_5H_4O_2$) and 3,4,5-trimethyl-2-cyclo-penten-1-one.

4. Column chromatography

A look at the chromatograms obtained from extracts of *J. procera* shows that similar components were obtained by both destructive distillation and Soxhlet methods. Therefore, it seems more appropriate to use the destructive distillation extraction method since it avoids the use of the expensive organic solvents, which are often toxic.

To confirm the components obtained above, column chromatography was performed on the crude extracts, aimed at effective isolation and characterization of the components. A packed column with silica gel gave five fractions from the dry distillation extracts of *J. procera*.

The isolated components were analysed by GC and GC/MS spectrometers. Not all the components detected in the crude extracts were isolated. Fractions F1 and F2 gave similar components as above. However, MS fragmentation pattern of F3 had the following *m/e*: 204 (molecular ion), 161, 119 (base peak), 105, 93, 77, 69, 55 and 41. Overall, this spectra matched that of cedrol. However, fraction F3 decolourized bromine water, though no reaction with Brady's reagent or Fehling's reagent, suggesting absence of carbonyl groups, but presence of olefin-like groups. The molecular ion corresponding to *m/e* 204 and existence of double bond suggested the compound to be cedrene (X6), other than cedrol, which was previously reported to occur together with cedrol in cedar oil (Wise, 1952). This was confirmed by IR spectra, which showed a peak beyond 3000 cm^{-1}, suggesting unsaturation. The bands between 1300 and 750 cm^{-1} suggest that the compound is not linear but rather ring-like. The UV-VIS spectra had an absorption maxima at 228 nm, suggesting unsaturation (Shriner et al., 1980). On the other hand, system 1 had no UV-VIS band beyond 200 nm and did not decolourize bromine water.

Column B was similar to column A, except that the eluting solvent volumes were different. Fraction F4 from column B gave a very prominent peak while GC/MS gave at a scan number 1343. The MS of F4 was similar to that of F1, except that F4 had an additional peak at *m/e* 248, suggesting a derivative of cedrol or copaene, FW 204, most likely a carboxylic acid derivative with the additional-CO_2 group to give FW 248.

5. Conclusions

From this work it was evident that the water content in the extract was 77.5%, whereas the oily layer was 22.5%. The isolated fractions contained cedrol, cedrene and possibly carboxylic acid derivative of cedrol or copaene, which were earlier found to have antitermite effects (Harris, 1971). Moreover, UV-VIS, IR and Mass spectra of the extracts indicated presence of other components, which included phenolic and alcoholic-like molecules. Some of the fractions could be byproducts as a result of decomposition during destructive distillation.

The presence of *m/e* 204 as the molecular ion other than *m/e* 222 for cedrol by GC/MS, suggests that cedrol enters GC/MS as cedrol minus H_2O molecule. The decomposition could have occurred during extraction procedures, which involve heating, or prior to entry into the spectrometer.

Gas chromatogram peak areas indicate that cedrol and cedrene were about 10% and 6%, respectively, of the total components in the isolated extract. These two compounds had been found to have antitermite activity. However, it was difficulty to characterize and quantitate all the 10 components revealed by TLC.

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