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Phytochemical and Anthelmintic Study of the Root Bark of *Teclea Trichocarpa*, Engl. (Rutaceae)

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The root bark of *Teclea trichocarpa* exhibited anthelmintic activity against egg hatching and larval development of sheep nematodes (*Strongyloides*). Three compounds, namely lupeol, melicopicine and 6-methoxytecleanthine were isolated from the dichloromethane-methanol (50:50) extract of the plant. Melicopicine and 6-methoxytecleanthine exhibited mild anthelmintic activity. The present study lends scientific credence to the traditional use of *Teclea trichocarpa* in the treatment of human helminth infections.

Key words: *Teclea trichocarpa*, phytochemical, anthelmintic

INTRODUCTION

Teclea trichocarpa is an evergreen much-branched shrub or tree growing to about 2-10 m in height with a smooth grey bark and short-hairy young branchlets. Its leaves are alternate, 3-foliolate, dark green and aromatic when crushed [1,2]. The tree is widely distributed in coastal and upland forests and grasslands, often near rivers. It also occurs in dune bush, forest edges as well as in lowland rain forests, riverine forests and dry semi-deciduous forests, especially in rocky localities, from sea-level up to 2300 m altitude. It is indigenous to Kenya where it is found in Ngong Forest (Kajiado county), Marakwet District and Keiyo District [1,3].

The Akamba people use the leaf extracts to treat malaria, fever and helminthiasis [3,4,5]. The vapour of leaves in hot water is inhaled to treat fever. The Giriama (Kenya) put the strong-smelling leaves in the nose of their hunting dogs to improve their scenting powers [4]. The stem bark infusion is taken to treat malaria. The smoke of green twigs on a fire is used to treat body pain and hepatitis. The leaf decoction is taken to treat pneumonia [1].

EXPERIMENTAL

Plant collection and identification

The root bark of *Teclea trichocarpa* was collected from Ngong Forest, Kajiado County, in October 2012. Taxonomical identification was done at the Department of Botany Herbarium, University of Nairobi and a voucher specimen deposited. The plant material was air-dried in the shade at room temperature and milled.

Preparation of extracts

The powdered plant material (924 g) was subjected to four consecutive cold maceration extractions using dichloromethane-methanol (1:1). The extracts were combined and dried *in vacuo* using a rotary evaporator to produce 77 g of dry extract which represented 8.3 % yield.

Isolation of compounds

About 32 g of dry extract was adsorbed on silica gel, loaded onto a column packed with 189 g silica gel and eluted using 100 % ethyl acetate. Fractions were collected in test tubes (F_1 to F_{589}) and monitored using TLC visualized using UV (254 and 366 nm), iodine and vanillin. The fractions were pooled based on TLC profiles to three major fractions; F1 (F_{54} - F_{63}), F2 (F_{81} to F_{110}) and F3 (F_{194} to F_{220}). The three fractions formed crystals under slow evaporation at room temperature.

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The crystals in fraction F1 were cleaned with acetone and re-crystallized in ethyl acetate to yield a pure white crystalline compound which produced a single TLC spot that was not visible in both short and long UV but was visualized in the iodine chamber. This compound was coded compound **1** and stored at 4 °C.

The crystals in fraction F2 were cleaned in n-hexane and re-crystallized severally in warm ethyl acetate to yield a pure yellow crystalline compound. The compound produced a single spot on TLC when visualized under long UV radiation (366 nm). This compound was coded compound **2** and stored at 4 °C.

The crystals in fraction F3 were cleaned with cold ethanol and re-crystallized in warm ethyl acetate to yield a pure compound that showed a single TLC spot when visualized under both short and long UV radiation. The compound was coded compound **3** and stored at 4 °C.

Egg hatch assay

The assay was carried out in in microtitre plates. Each compound was dissolved in a dimethylsuphoxide-water (3:97) solution and serially diluted to give a concentration range of 39.0625 to 5,000 µg/ml. Twenty µl aliquots of the serially diluted compounds were transferred to test wells while 20 µl of DMSO was transferred to negative control wells. About 55 nematodes eggs (*Strongyle* species) were transferred to each well. The final volume of the wells was made to 80 µL using distilled water. The plates were incubated for 48 hours. The number of hatched larvae and the number of eggs remaining in each well was counted and the percentage inhibition of hatching at each concentration determined.

Larval development assay

Nematode eggs (about 55 eggs in 80 µL per well) were incubated in microtitre plates for 48 h at 27 °C, conditions conducive for egg hatching. To each well, 20 µL of the pure compound (5000 µg/ml) was added to give a final concentration of 1000 µg/ml and the mixture incubated for a further five days to allow the larvae to develop. The number of larvae that did not develop were counted and the percentage inhibition of development calculated.

RESULTS AND DISCUSSION

Phytochemical tests

The root bark of *Teclea trichocarpa* was found to contain alkaloids and tannins but no glycosides.

Structure elucidation of isolated compounds

The structures of isolated compounds are shown in Figure 1.

Compound 1 (Lupeol)

Isolated as white feathery crystals; m.p. 211-213 °C; IR (KBr) cm^{-1}: 3393 (O-H stretch), 2943 (=C-H stretch), 2868 (aliphatic C-H stretch), 1638 (C=C stretch) and 881 (out of plane C-H bend); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 4.57 (d, *J*=2.4 Hz, 2H, H-29), 3.20 (m, 1H, H-3), 2.32 (m, 1H), 1.86 (m, 1H), 1.68 (s, OH), 1.63 (s), 1.58 (s, H-30), 1.53 (m), 1.41 (d, *J*=4 Hz, H-28), 1.37 (s, H-27), 1.36 (s, H-26), 1.30 (s), 1.28 (s, H-25), 1.25 (s, H-24), 1.03 (s, H-23), 0.95 (d, *J*=4 Hz), 0.83 (s), 0.77 (d, *J*=8 Hz), 0.67 (d, *J*=8 Hz); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 38.9 (C-1), 27.7 (C-2), 79.2 (C-3), 39.1 (C-4), 55.5 (C-5), 18.5 (C-6), 34.5 (C-7), 41.1 (C-8), 50.7 (C-9), 37.3 (C-10), 21.1 (C-11), 25.4 (C-12), 38.2 (C-13), 43.1 (C-14), 27.7 (C-15), 35.8 (C-16), 43.2 (C-17), 48.5 (C-18), 48.2 (C-19), 151.2 (C-20), 29.9 (C-21), 40.2 (C-22), 28.2 (C-23), 15.6 (C-24), 16.3 (C-25), 16.2 (C-26), 14.8 (C-27), 18.2 (C-28), 109.6 (C-29) and 19.5 (C-30); EI-MS (*m/z*): 426 (100%) [M^+], 411 (53.4%), 383 (15.8%), 365 (20.9%) and 344 (9.4%).

Compound 2 (Melicopicine)

Isolated as yellow crystals; m.p. 128-130 °C; UV λ_{max} (Acetonitrile) nm: 267 and 401; IR (KBr) cm^{-1}**:** 3420, 3263 (O-H stretch of the enol form), 2995, 2940, 2868 and 2843 (C-H stretch), 1634 (C=O stretch of the cyclic α,β-unsaturated ketone), 1591 (N-H bend), 1122 (C-O stretch) and 980 (out of plane C-H bend); ^{1}H-NMR (200

MHz, $CDCl_3$) δ 8.31 (d, *J*=9 Hz, H-8), 7.36 (d, *J*=9 Hz, H-7), 7.65 (m, H-6), 7.15 (m, H-5), 4.09 (s, 3H, H-11), 3.98 (s, 3H, H-12), 3.96 (s), 3.93 (s, 3H, H-13), 3.88 (s, 3H, H-14), 3.75 (s, 3H, H-15) and 3.09 (s); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 152.7 (C-1), 145.1 (C-2), 142.1 (C-3), 150.4 (C-4), 116.1 (C-4a), 115.8 (C-5), 121.5 (C-6), 127.2 (C-7), 133.3 (C-8), 139.1 (C-8a), 177.5 (C-9), 137.5 (C-9a), 124.4 (C-10a), 62.2 (C-11), 62.0 (C-12), 61.9 (C-13), 61.7 (C-14) and 42.0 (C-15); EI-MS (*m/z*): 329 (24.9%) [M^+], 314 (100%), 299 (7%), 286 (6.9%), 284 (38.4%), 271 (37.4%) and 256 (75.1%).

Compound 3 (6-methoxytecleanthine)

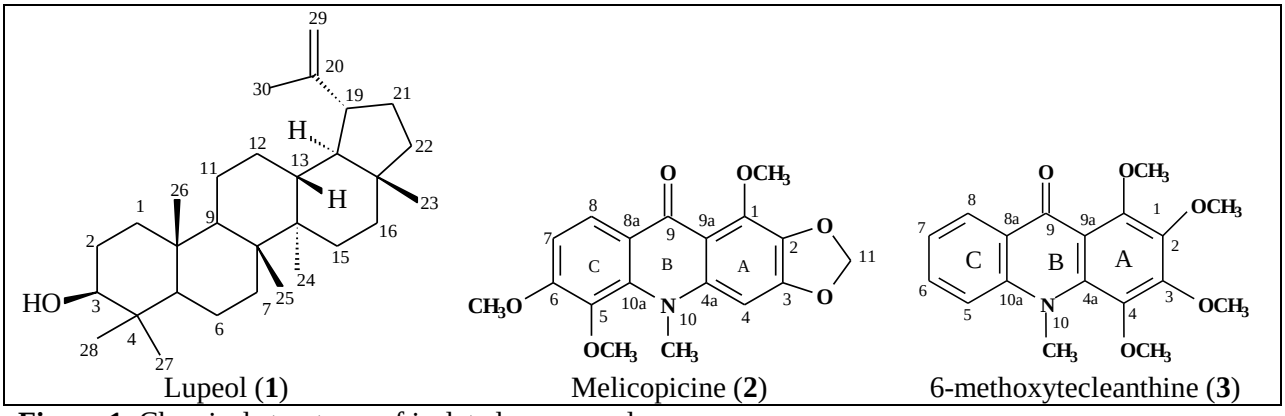
Isolated as green feathery crystals; m.p. 163-165 °C; UV λ_{max} (acetonitrile) nm: 272 and 391 nm; IR (KBr) cm^{-1}: 3451 (O-H stretch of the enol form), 2943 (aromatic C-H stretch), 2851, 2628 (aliphatic C-H stretch), 1636 (C=O stretch of the cyclic α,β-unsaturated ketone), 1601 (C=C stretch), 1231 (C-O stretch) and 1060 (out of plane C-H bend); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.18 (d, *J*=9 Hz,1 H, H-8), 6.90 (d, 1H, H-7), 6.64 (d, *J*=9 Hz, 1H, H-4), 6.01 (s, 2H, H-11), 4.13 (s, 3H, H-12), 3.98 (s, 3H, H-13), 3.89 (s, 3H, H-14) and 3.78 (s, 3H, H-15); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 156.7 (C-1), 137.0 (C-2), 138.3 (C-3), 91.1 (C-4), 121.5 (C-4a), 145.4 (C-5), 153.6 (C-6), 107.6 (C-7), 112.3 (C-8), 143.0 (C-8a), 177.0 (C-9), 123.6 (C-9a), 132.9 (C-10a), 101.9 (C-11), 61.3 (C-12), 41.9 (C-13), 56.5 (C-14) and 61.2 (C-15); MS (*m/z*): 343 100% [M^+], 328 (41.7%), 315 (77.2%), 300 (84%), 298 (77.6%), 283 (68.3%), 269 (57.3%) and 255 (47%).

Egg hatch assay

Melicopicine, 6-methoxytecleanthine and the crude extract had IC_{50} values of 509.28 µg/ml, 352.29 µg/ml and 185.25 µg/ml, respectively. Lupeol did not show any activity over the test concentration range.

Larval development assay

The IC_{50} values for the three pure compounds were above 1000 µg/ml and the compounds were thus considered inactive.



Lupeol (**1**) Melicopicine (**2**) 6-methoxytecleanthine (**3**)

Figure 1: Chemical structures of isolated compounds

CONCLUSION

In this study, three compounds, namely lupeol, melicopicine and 6-methoxytecleanthine were isolated from the dichloromethane-methanol (1:1) extract of the root bark of *Teclea trichocarpa*. This is the first report of the isolation of lupeol from this plant. Lupeol has previously been isolated from the related species *Teclea nobilis* [7]. Melicopicine and 6-methoxytecleanthine have been previously isolated from other parts of this plant. Melicopicine and 6-methoxytecleanthine exhibited weak anthelmintic activity by inhibiting the hatching of the *Strongyloides* eggs. Lupeol did not exhibit anthelmintic activity. All the pure compounds and the crude extract of the root bark of *Teclea trichocarpa* exhibited no activity against the development of the *Strongyloides* larvae. Further work should be

done to identify more active compounds from the plant and to screen the crude extract and the isolated compounds for other biological activities including anthelmintic activity on cestodes and trematodes.

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