Short Communication

Isolation of the anti-bacterial vernodalin from traditionally used Vernonia colorata

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Leaf extracts of Vernonia colorata were screened for anti-bacterial activity using the disc-diffusion assay. Micrococcus luteus, Klebsiella pneumoniae and Staphylococcus aureus were inhibited by ethyl acetate extracts, but not by hexane and water extracts. The ethyl acetate extract was fractionated by silica vacuum chromatography and a chromatron using hexane:ethyl acetate gradients. The presence of anti-bacterial activity was confirmed by bioautography. The major anti-bacterial compound in the ethyl acetate leaf extract was identified by NMR as vernodalin. The minimal inhibitory concentration of vernodalin against S. aureus was 100μg ml⁻¹.

The genus Vernonia belongs to the family Asteraceae. It is mainly found in tropical areas, principally Africa. The common name of V. colorata (Wildoewr) Drake is the lowveld tree vernonia or lowveld bitter tree. Ibozane being its Zulu name (Hutchings et al. 1996). It is an erect shrub or small tree that grows up to five metres high. The species is widespread in west, central and south tropical Africa, extending southwards through Mpumalanga, Swaziland and Mozambique to northern Kwa-Zulu Natal.

Decoctions of leaves of V. colorata are used traditionally against fevers and as expectorants and laxatives. The bark of roots and stems are used for fever and diarrhoea. Roots are used as a tonic, to treat boils, fevers and as a cough remedy (Hutchings et al. 1996). V. colorata is used by traditional medical practitioners in Zimbabwe to treat abdominal pains, bilharziasis, rheumatism, fever, infertility in women, weak joints, oedema and a painful uterus (Gelfand et al. 1985). Extracts of V. colorata are reported to have anti-normoebic, anthelminthic, anti-bacterial and parasympatholytic properties, but no anti-inflammatory activity (Hedberg and Staugard 1989, Kelmanson et al. 1999).

The known chemical constituents and biological properties of V. colorata include: vernonin which has been isolated from roots (Patel and Rawson 1964), two sesquiterpene lactones with in vitro cytotoxic action (Hedberg and Staugard 1989) and vernolide, with anti-parasitic (Koshimura et al. 1993) and anti-inflammatory (Satoda and Yoshi 1962, Benoit 1976) activities. Compounds with anti-inflammatory properties including an unidentified sesquiterpene lactone and glycosides have been found in flowers and bark of V. colorata (Satoda and Yoshi 1962, Benoit 1976). A cardiac glycoside was isolated from stems, leaves and roots of V. colorata in Nigeria (Patel and Rawson 1964). Significant anti-malarial activity was observed for lipophilic extracts of V. colorata (Kraft et al. 1999).

We report here on the screening of the leaves of Vernonia colorata for anti-bacterial activity to determine if the use of V. colorata leaves for bacteria related diseases can be substantiated. The subsequent isolation of the major anti-bacterial compound present in the ethyl acetate leaf extract is outlined. Leaves were collected from a tree of Vernonia colorata growing in the medicinal garden of the University of Natal Botanical Garden, Pietermaritzburg. A voucher specimen is deposited in the University of Natal Herbarium (Kelmanson-4UN). Plant material was dried in an oven at 50°C for 72h. The dried material was then ground and stored in the dark at room temperature until further processing.

Plant extracts (hexane, ethyl acetate, water and ethanol) were initially tested for anti-bacterial activity using the disc-diffusion assay (Rasanoairo and Ratisimamanga-Urverg 1993). Extracts were tested against seven strains of bacteria obtained from the Microbiology Department of the University of Natal, Pietermaritzburg. The bacteria used were: Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa (Gram-negative) and Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus and Staphylococcus epidermidis (Gram-positive). Stock cultures of bacteria were stored at 4°C on nutrient agar plates. Neomycin was used as a positive control. The neomycin was diluted to 200μg ml⁻¹, and 10μl then pipetted onto each disc.
For bioassay guided fractionation the bioautographic assay (Slusarenko et al. 1989) was used to determine the areas of anti-bacterial activity on a TLC plate. A TLC plate containing the plant extract was run with the solvent ratio of hexane: ethyl acetate 1:1 (v/v). An overnight culture of S. aureus was grown in Mueller-Hinton (MH) broth, in a water bath at 35°C. The overnight cultures were used to inoculate new broth cultures (15ml). After 5-6h, the bacterial cells were centrifuged at 3000g for 10min at room temperature. The pellet was re-suspended in 2ml of the broth. The overlay medium consisted of 15ml of Mueller-Hinton (MH) agar, supplemented with 15mg 2,3,5-triphenyltetrazolium chloride. The bacterial suspension was added to the agar overlay medium and the mixture was immediately poured over the whole TLC plate, in an even layer. The solidified overlay was placed on damp paper-roll in a metal tray, and sealed with plastic film, to ensure that the overlay remained moist during overnight incubation at 37°C.

Anti-bacterial activity was only present in the ethyl acetate extract. Three bacterial strains were inhibited by the extract: M. luteus (0.53), S. aureus (1.40) and K. pneumoniae (0.53). The highest inhibition was obtained against Gram-positive bacteria. Gram-negative P. aeruginosa and E. coli strains were not inhibited by the ethyl acetate leaf extract.

For the identification of the major active compound ground leaf material (205g) was defatted by Soxhlet with hexane (21). Further extraction of the defatted residue was carried out by Soxhlet with ethyl acetate (21). The ethyl acetate extract (35.3g) was taken to dryness and re-suspended in ethyl acetate (100mg ml-1). Part of this extract (3g) was fractionated on a vacuum silica gel column (Silica gel 60: 0.040–0.063 mm) and eluted with a linear hexane: ethyl acetate gradient (10:0–0:10). Four hundred ml of solvent mixture was used. Each fraction was taken to dryness under vacuum and the yield of each fraction determined. Activity was detected in the fraction collected during the elution with the hexane:ethyl acetate ratio of 160:240ml (v/v). This fraction was separated on a chromatron (Silica gel 60 PF254 containing CaSO4). The active fraction was applied to a 1mm thick chromatron plate and eluted with a gradient of hexane:ethyl acetate (10:0; 8:1; 8:2; 1:1) (v/v). One hundred ml of each solvent mixture were used. Overall, twelve fractions were collected from the chromatron. A bioautographic assay showed the presence of anti-bacterial activity in the fraction that eluted from the chromatron with hexane:ethyl acetate 1:1 (v/v).

The isolated biologically active compound was analysed by NMR in a solution of methanol. The 13C-NMR spectrum of the sample dissolved in CD3OD was recorded at 200 MHz using a Kratos MS 80RF double-focusing magnetic sector instrument at 70eV. TMS was used as an internal standard. The 13C-NMR data for the compound with anti-bacterial activity was: 13C-NMR (50 MHz, CH3): δ 41.0 (C-9), 42.9 (C-10), 47.5 (C-5), 51.8 (C-7), 62.0 (C-19), 70.0 (C-8), 72.0 (C-14), 79.8 (C-6), 117.4 (C-2), 121.5 (C-13), 126.8 (C-18), 133.0 (C-4), 135.8 (C-11), 135.8 (C-15), 135.8 (C-17), 141.8 (C-1), 166.2 (C-16), 166.4 (C-3), 171.0 (C-12).

From analyses of the NMR spectrum and comparison to published NMR-data (Al Magboul et al. 1997) it was identified as vernoladin (Figure 1). This is the first identification of vernoladin in V. colorata.

Minimal inhibitory concentration (MIC) for the isolated compound was determined using a serial dilution technique (Eloff 1998). An overnight culture of S. aureus was grown in MH broth, in a water bath at 35°C. The following morning, 100μl of the compound solution was added to the first well of a 96 well microtiter plate. The compound was serially diluted with water. The overnight culture was diluted 1/100 with sterile MH broth, and 100μl was added to each well. The microplate was covered with parafilm and incubated at 37°C overnight. On the final day, 40μl of 0.2mg ml-1 p-nitrotetrazolium violet was added to each well and the microplate was covered and incubated at 37°C for 30min. A MIC of 100μg ml-1 was determined for vernoladin against S. aureus.

Vernoladin was quoted in Zdero et al. (1991) to have been isolated and identified from V. colorata, but no structural data was given, and the data referred to were for vernoldine (Toubiana and Gaudemer 1967). Ho and Toubiana (1969) and Pascard (1970) referred only to the compound vernoldine. Harborne and Williams (1977) mentioned the presence of vernolide and hydroxyvernolide in V. colorata, and referred to the presence of vernoladin only in V. amygdalina. Vernoladin is known to have anti-tumor, anti-fungal and anti-bacterial activity. Vernolepin and vernoladin, at a concentration of 10μg ml-1, showed anti-bacterial activity similar to that of ampicillin and neomycin at a 400μg ml-1 concentration (Al Magboul et al. 1998). Vernoladin showed fungicidal activity at a concentration of 10μg ml-1 against Aspergillus niger and a fungistatic activity against Candida albicans (Al Magboul et al. 1998). Our results therefore substantiates the use of V. colorata for bacteria related diseases.

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Figure 1: Structure of vernoladin, the major anti-bacterial compound isolated from Vernonia colorata leaves.
References


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