

## The *in vitro* Anti-Fungal and Anti-Bacterial Activities of $\beta$ -Sitosterol from *Senecio lyratus* (Asteraceae)

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*Senecio lyratus*,  $\beta$ -Sitosterol

From a methanol extract of dried-ground aerial parts of *Senecio lyratus*, an anti-fungal and anti-bacterial active compound was isolated and identified as  $\beta$ -sitosterol by spectroscopic analysis.

### Introduction

The genus *Senecio* (Asteraceae) has been widely investigated and nearly all species contain pyrrolizidine alkaloids (PA) as the most characteristic metabolites (Bull, 1968; Bick, 1985; Mattocks, 1986; Rizk, 1991; Bottcher *et al.*, 1993; Vrieling *et al.*, 1993; Obuya *et al.*, 1993; Grue and Liddell, 1993; and Cheng *et al.*, 1993). PA's have been found to possess interesting medicinal properties, some are carcinogenic while others have been reported to exhibit anti-tumour and other activities (Mattocks, 1986 and Rizk, 1991). However, there has been no investigation on the Kenyan *Senecio lyratus* (Asteraceae). Traditionally the plant is used in the treatment of wounds and as an emetic (Kokwaro, 1976). Some highland people of Kenya particularly the Kipsigis and the Maasai are known to use the plant for the treatment of venereal diseases (information from traditional medicine men). The plant is found in the Savannah vegetation throughout Africa. In Kenya it is distributed in altitudes between 1500 m and 2760 m above sea level and commonly found in upland forests and woodland areas around Mt. Kenya, Cherangani highlands, Mau forest, Kericho district and the Kisii highlands among other regions (Agnew and Shirley, 1994). It is a creeping wiry trailing climber, long hairy on all young plants with triangular or ovate toothed leaves (Kokwaro, 1976). In the pre-

sent study, we have been interested in the biologically active principles of this species. Thus, this communication describes the isolation of  $\beta$ -sitosterol (**1**) from *Senecio lyratus* and assay results based on anti-fungal and anti-bacterial activities.

### Material and Methods

#### General

Plant material was collected from Kericho district in March 1997 and authenticated by a herbarium staff of the University of Nairobi where a voucher specimen has been deposited. The aerial parts of the plant were dried in a shade (away from direct sunlight) and later ground into fine powder (1 kg) which was soaked in 1 l of methanol and left standing for five days at room temperature. The resulting crude extract, which was obtained after the removal of solvent (using a vacuum rotary evaporator), was subjected to partition chromatography using *n*-hexane, dichloromethane and methanol successively to afford three fractions. The dichloromethane fraction was then passed through a column of silica gel ( $\varnothing = 5.5 \times 68$  cm) followed by separation using several preparative thin layer chromatography (TLC) to give a white crystalline solid (125 mg, 0.0125% yield w/w) recrystallized in acetone (Mp. 130 °C). Spot tests on the compound gave a blue color with Liebermann-Burchard reagent, a positive test for sterols. The compound was identified as  $\beta$ -sitosterol (**1**), *m/z* 414, (C<sub>29</sub>H<sub>50</sub>O) on the basis of spectroscopic data.

<sup>1</sup>H NMR spectra were recorded at 400 MHz on a Bruker Avance 400 MHz instrument of the University of Botswana. MS was by Electron Impact (EI, solid probe 7.01 e6) on VG 12–250 UPGRADED instrument at the International Center for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya while the IR was performed at Kenyatta University on a PERKIN-ELMER, 598 spectrophotometer. The removal of solvents which was on BIBBY-Rotary Evaporator RE-100 and melting points (which are uncorrected) including UV analysis were all done at Jomo Kenyatta University of Agriculture and Technology (JKUAT). Column chromatography was done on silica gel 60 (230–400 mesh) while TLC was with Kieselgel 60



F<sub>254</sub> (Merck 5554, 0.2 mm) on aluminium pre-coated plates. Nutrient agar (peptone 5.0 g/l, beef extract 3.0 g/l, sodium chloride 8.0 g/l and agar No. 2, 12.0 g/l at pH 7.3 ± 0.2) and potato dextrose agar (potato extract 4 g/l, glucose 20.0 g/l and agar 15.0 g/l at pH 5.6 ± 0.2) plus all solvents were obtained commercially. The anti-bacterial and anti-fungal tests were done in the Department of Botany, JKUAT.

### Bioassays

#### Anti-bacterial assay test

In this test, paper disc method was adopted (Brooks *et al.*, 1991). It involved sub-culturing bacteria from a bacteria stock into sterilized nutrient broth for 24 hours and then inoculating them into

nutrient agar contained in a petri dish. A blotting/ filter paper cut out so as to have protruding parts, was placed carefully into the petri dish. Around the tips of the protruding parts of the paper, 20 µl of 5 mg/ml stock solution (i.e. 100 µg = 12 mm) of β-sitosterol (**1**) in *n*-hexane was uniformly applied together with a control in which 20 µl of *n*-hexane alone was used. All the experiments were done in triplicate. The set-ups were run at 37 °C and the results obtained after 48 h.

Positive test results – an indication of growth inhibition were obtained when no bacterial growth were observed around and close to the tips of the protruding parts while negative test results were observed when there was growth around these regions like the corresponding control. The bacteria used for the assay were *Salmonella typhi*, *Corynebacterium diphtheriae*, *Bacillus subtilis*, *Shigella*

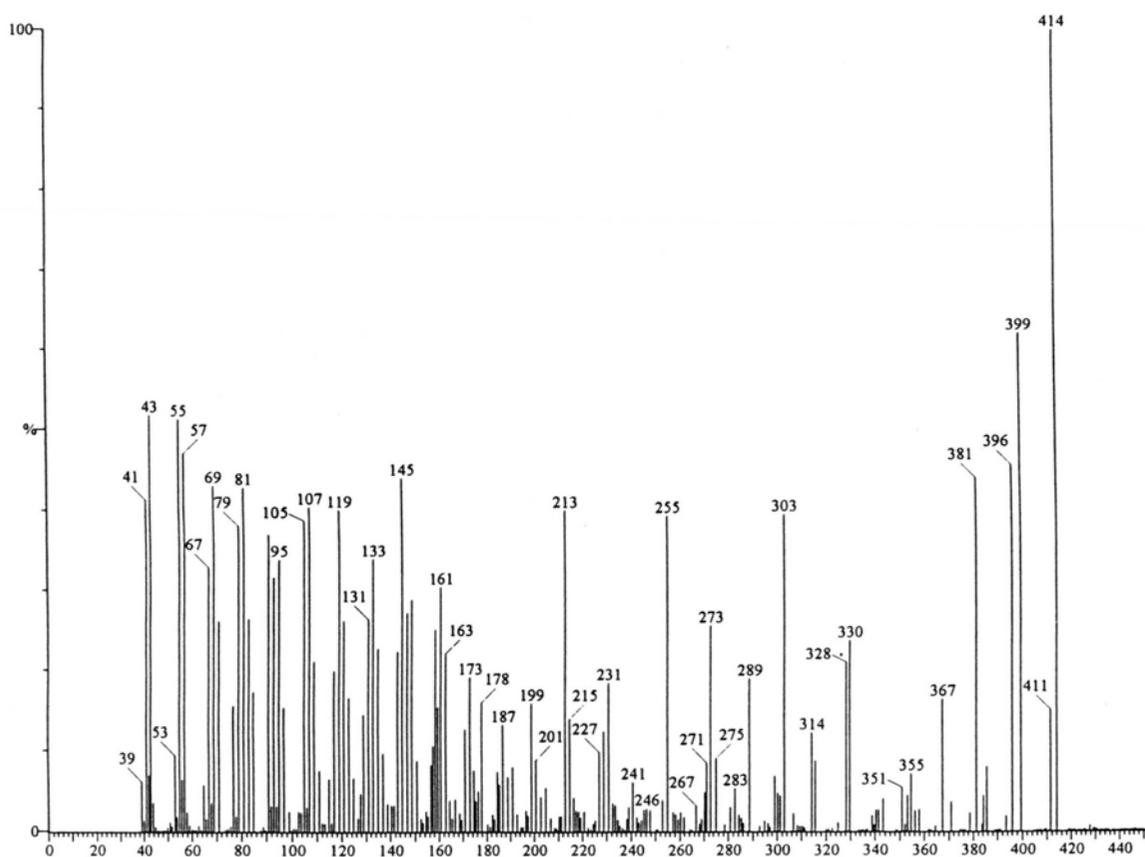


Fig. 1. MS Spectrum of β-sitosterol (**1**) done on a VG 12–250 URGADED instrument solid probe (EI).

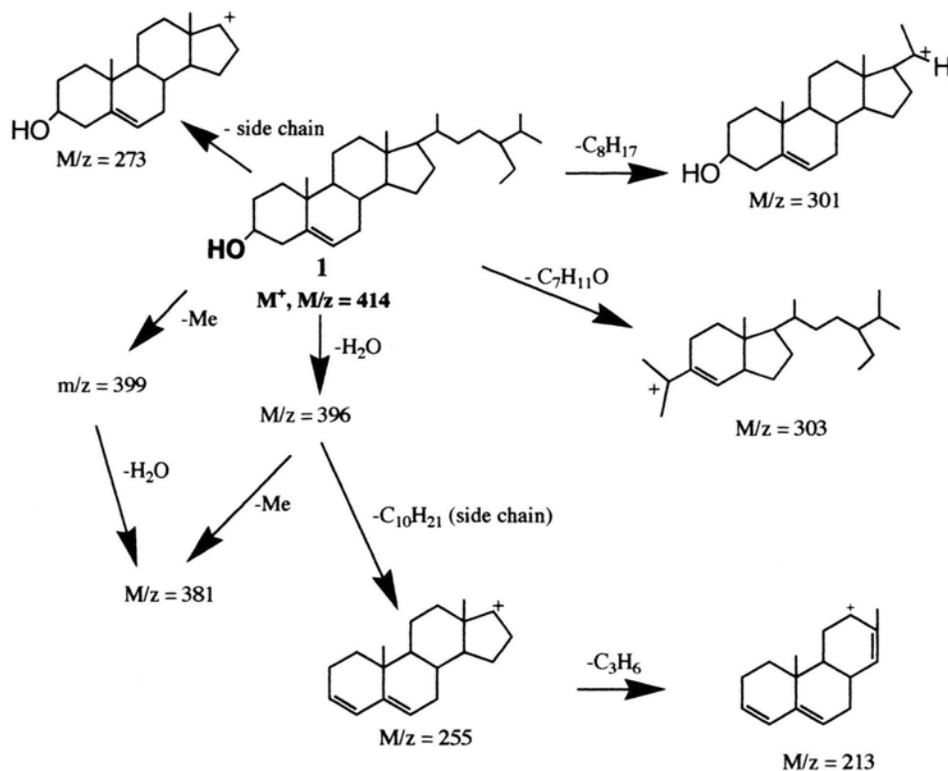


Fig. 2.  $\beta$ -Sitosterol (**1**) – MS fragmentation pattern showing the various fragments that are responsible for the most prominent peaks seen in Fig. 1.

*dysenteriae* and *Vibrio cholerae*. The results of these tests are summarized in Table IA.

#### Anti-fungal assay test

A similar experimental procedure for the anti-fungal tests was followed like the anti-bacterial tests above. The differences being on the growth medium used and the period of observation. In this case, potato dextrose agar was used and that the experiment was followed for four days instead of 48 h. The fungi used were *Fusarium spp.* and *Penicillium spp.* The results of these tests are summarized in Table IB.

#### Results and Discussions

$\beta$ -Sitosterol (**1**), C<sub>29</sub>H<sub>50</sub>O, was obtained as colorless crystals, Mp. 130 °C recrystallized in acetone. EI-MS spectrum (Fig. 1) showed a molecular ion peak at  $m/z$  414. Spot test on the compound gave a

blue color with Liebermann-Burchard reagent. MS fragmentation pattern (Fig. 2) was in agreement with the observed prominent peaks that are seen in Fig. 1. In the <sup>1</sup>H NMR spectrum, the OH peak was seen at  $\delta$  5.4, the methylene (unsaturated carbon) proton was quite discernible at  $\delta$  4.2 (*triplet*) and a H-C-O proton system was observed at  $\delta$  3.6 (*multiplet*) while the rest of the proton systems were catered for by several peaks clearly seen between  $\delta$  0.7 and  $\delta$  2.4. Using this data and comparing with literature (Tsanuo, 1992), the compound was identified as  $\beta$ -sitosterol (**1**). It has also been isolated from *Vernonia galamensis ssp. nairobiensis* (Mwaura *et al.*, 1996). Anti-bacterial test results indicated  $\beta$ -sitosterol (**1**) at 12 mM to be active against *Salmonella typhi* and *Corynebacterium diphtheria* (Table IA). Although its activity against *Vibrio cholerae* was comparatively small, the mere fact that it showed some activity against it, is a clear indication of its potency. Moreover, it has to be considered

Table I. A. Effects of  $\beta$ -sitosterol (**1**) [12 mM] on various species of bacteria after 48 hr.  
 B. Effects of  $\beta$ -sitosterol (**1**) [12 mM] on *Fusarium spp.* and *Penicillium spp.* after 4 days.

A.	
Bacteria species	Average inhibition diameter [mm]
<i>Salmonella typhi</i>	3.0
<i>Corynebacterium diphtheriae</i>	3.0
<i>Bacillus subtilis</i>	2.0
<i>Shigella dysenteriae</i>	< 1.0
<i>Vibrio cholerae</i>	< 1.0
Control	0.0

B.	
Fungi species	Average inhibition diameter [mm]
<i>Fusarium spp.</i>	10.0
<i>Penicillium spp.</i>	0.0
Control	0.0

that *V. cholerae* is very resistant to antibiotics. Anti-fungal test results showed  $\beta$ -sitosterol (**1**) also at 12 mM to be very active against *Fusarium spp.* (average inhibition diameter of 10 mm) but inactive against *Penicillium spp.* (Table IB). This difference in activities in this case could be attributed to the morphological differences between the two fungi with *Penicillium spp.* being more resistant to attack by chemicals than *Fusarium spp.* This aspect of resistance of *Penicillium spp.* is further supported by the fact that it easily invades different

niches. Thus, the biological activities observed here with comparatively high concentrations are interesting phenomena with regard to the isolation and development of drugs from Kenyan plants and in particular from *Senecio lyratus* whose systematic investigation is being done for the first time. The activities seen in this investigation are not unique with  $\beta$ -sitosterol (**1**) since it was also found to show weak feeding inhibitory activities against the larvae of *Chilo partellus* (Tsanuo, 1992).

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