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Phytochemical and Antimicrobial Investigation of *Girardinia diversifolia* (Link) Friis (Urticaceae)

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Root and stem extracts of *Girardinia diversifolia* exhibited varying degrees of activity against *Bacillus pumilus, Staphylococcus aureus, Escherichia coli, Aspergillus niger, Candida albicans* and *Saccharomyces cerevisiae*. Three compounds namely β-sitosterol, 7-hydroxysitosterol and 3-hydroxystigmast-5-en-7-one, were isolated from the petroleum ether root extract. The present study gives scientific credence to the traditional use of *Girardinia diversifolia* in the management of microbial infections.

Key words: *Girardinia diversifolia*, phytochemical, antimicrobial

INTRODUCTION

Girardinia diversifolia (Link) Friis (Urticaceae) is also referred to as giant nettle. It is an erect annual herb reaching 75-300 cm in height depending on soil nutrients and rainfall. It has large, up to 8 cm wide, lobed, coarsely dentate, alternate leaves. The stem grows up to 4 cm in diameter at the base. The flowers are monoecious or dioecious occurring in dense axillary inflorescences, while the roots are fibrous. The leaves, stems and flowers are covered with long, slender stinging trichomes [1,2].

The leaf of *G. diversifolia* is used externally as an astringent and in treatment of scrofula [3]. A decoction of the roots and basal stems is used orally as a cure for malignant boils, gastric problems and constipation [4]. The root is applied externally for treatment of cuts and wounds, headaches and swollen joints [5]. A decoction of the plant is used to treat fevers while its ashes are applied externally in the treatment of ringworms and eczema.

EXPERIMENTAL

Plant collection and identification

The roots and stems of *G. diversifolia* were collected from Kamweti Location neighbouring Mt. Kenya Forest, Kirinyaga District, Kenya, in September 2006. The plant was identified and authenticated at site by a taxonomist. A specimen of the plant is deposited in the herbarium of the Department of Botany, University of Nairobi (voucher specimen number SoP/06/001). The collected roots and stems were dried at room temperature and ground.

Preparation of extracts

The root and stem powders were sequentially Soxhlet extracted with petroleum ether (60-80 °C), chloroform and methanol, and the resultant extracts reduced to dryness *in vacuo*. The methanol extract was partitioned between water and ethyl acetate to give the ethyl acetate extract.

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Isolation of compounds from the petroleum ether root extract

The petroleum ether root extract was fractionated on silica gel by gradient elution from benzene to chloroform to give eleven fractions. Recrystallization of fraction F8, F9 and F10 in acetone yielded colourless plates (I), colourless needles (II) and small colourless plates (III), respectively.

Screening for antibacterial and antifungal activities of *Girardinia diversifolia*

The agar diffusion assay method was used. The test microorganisms were subcultured overnight for 18 h in their prescribed nutrient media to obtain working cultures. A 50 mg/ml solution of each extract was prepared in dimethyl sulfoxide. Test solutions of standard antibiotics were also prepared thus: gentamicin 10 µg/ml, benzylpenicillin 1 IU/ml and nystatin 150 µg/ml according to Hewitt and Vincent protocol [6].

Nutrient media for growth of the test microorganisms were prepared as per the manufacturer′s instructions, autoclaved at 125°C and cooled to 50 °C. Each of the cultured microorganisms was suspended in 5 ml of sterilized distilled water and approximately 5 ml of suspension inoculated into respective growth medium to produce agar with approximately 1×10^6 colony forming units per milliliter. The inoculated nutrient media were then rapidly but carefully poured into petridishes in such a manner as to deliver 20 ml of agar with a uniform thickness of 3 mm in each petridish. The layered agar was allowed to cool and set into a firm gel suitable for plating-out procedure.

Using a symmetric paper template with six circles drawn in a hexagonal array, six cylindrical wells were punched into the layered media using a cork borer. The sample extracts were applied into the wells using a fixed-volume micropipette set to deliver 50 µl per well. Each extract was applied in duplicate. Dimethyl sulfoxide and standard antibiotics as appropriate were included in each petridish as negative and positive controls respectively.

A pre-diffusion period of 1 h was allowed to facilitate diffusion of the applied solutions into the inoculated media before the petridishes were incubated for 18 h at 37 °C. The diameters of the zones of inhibition were measured and preserved by photography.

Bioautography

The test microorganisms, *B. pumilus* and *S. cerevisiae*, were incubated for 18 h at 37 °C. Tryptone soy agar and Sabouraud′s dextrose agar nutrient media were prepared as per the manufacturer′s instructions. A 20 mg/ml solution of each extract was prepared by dissolving 100 mg of petroleum ether, chloroform and ethyl acetate extracts in 5 ml of chloroform, and the methanol extracts in 5 ml of methanol. A 100 µl aliquot of each solution was then spotted on each of the two glass-backed TLC plates, and the plates developed using a $CHCl_3$:MeOH, 90:10% v/v mobile phase. A 100 µl aliquot of 10 µg/ml gentamicin solution was applied on one plate, while to the other was applied 100 µl of 150 µg/ml nystatin solution.

A standardized inoculum of *B. pumilus* was inoculated uniformly into the prepared tryptone soy agar at 50 °C and the inoculated agar rapidly but carefully layered over the developed TLC plate to produce a uniform 1 mm thick agar layer. This procedure was repeated using nystatin-containing TLC plate and Sabouraud′s dextrose agar inoculated with *S. cerevisiae.* A pre-diffusion period was allowed by leaving the plates standing for 1 h at room temperature before incubation at 37 °C for 18 h.

The bioautograms were sprayed with a solution of methylthiazolyl tetrazolium bromide, followed by further incubation at 37 °C for 4 h and thereafter sprayed with absolute ethanol to kill the microorganisms. The bioautograms were observed for bioactive constituents identified by their R_f values and preserved by photography.

RESULTS AND DISCUSSION

Structural elucidation of the isolated compounds

The structures of the isolated compounds I, II and III are shown in Figure 1.

β-Sitosterol (I): UV λ_{max} (MeOH) nm: 208.5; IR ν_{max} (KBr) cm^{-1}: 3426 (O-H str), 2948 (CH_3, C-H str), 2876 (CH_2, C-H str), 1454 (CH_3, C-H bend), 1369 (=C-H bend); LRMS m/z (rel. int.): 415 ($(M+1)^+$, 100), 400 (31), 397 (47), 386 (3), 382 (29), 368 (3), 330 (51), 302 (3), 274 (24), 256 (26), 214 (36), 160 (26), 145 (32), 107 (38), 95 (32), 81 (35), 69 (28), 57 (31), 43 (82); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 5.35 (1H, d, H-6), 3.51 (1H, m, H-3), 2.27 (1H, d, H-4), 1.96 (1H, q, H-8), 1.92 (2H, t, H-1), 1.80 (2H, t, H-12), 1.74 (1H, br, s, H-25), 1.60 (2H, m, H-2), 1.57 (2H, t, H-7), 1.52 (2H, m, H-22), 1.33 (1H, m, H-25), 1.28 (2H, q, H-16), 1.24 (2H, m, H-23), 1.19 (2H, m, H-15), 0.99 (3H, s, H-19), 0.89 (2H, m, H-11), 0.83 (6H, m, H-26, H-27), 0.81 (3H, d, H-21), 0.78 (3H, m, H-29), 0.66 (3H, s, H-18); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 37.46 (C-1), 31.85 (C-2), 72.02 (C-3), 42.49 (C-4), 140.95 (C-5), 121.95 (C-6), 32.11 (C-7), 32.11 (C-8), 50.32 (C-9), 36.71 (C-10), 21.29 (C-11), 39.98 (C-12), 42.49 (C-13), 56.97 (C-14), 24.51 (C-15), 28.47 (C-16), 56.25 (C-17), 12.19 (C-18), 19.24 (C-19), 36.36 (C-20), 18.99 (C-21), 34.14 (C-22), 26.24 (C-23), 46.02 (C-24), 29.33 (C-25), 20.05 (C-26), 19.61 (C-27), 23.26 (C-28), 12.07 (C-29).

3-Hydroxystigmast-5-en-7-one (II): UV λ_{max} (MeOH) nm: 238.5; IR ν_{max} (KBr) cm^{-1}: 3413 (O-H str), 2947 (CH_3, C-H str), 2877 (CH_2, C-H str), 1661 (C=O str), 1458 (CH_3, C-H bend), 1347 (=C-H bend); LRMS m/z (rel. int.): 429 ($(M+1)^+$, 100), 414 (4), 396 (21), 386 (1), 372 (3), 288 (14), 248 (9), 235 (4), 214 (3), 256 (26), 206 (11), 193 (31), 188 (16), 174 (7), 161 (24), 135 (18), 107 (11), 95 (10), 81 (10), 69 (9), 57 (10), 43 (13); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 5.7 (1H, s, H-6), 3.7 (1H, m, H-3), 3.1 (1H, t, H-8), 2.6 (2H, m, H-4), 2.5 (1H, q, H-9), 2.4 (1H, q, H-24), 2.2 (2H, d, H-17), 2.0 (2H, m, H-12), 1.60 (1H, d, H-20), 1.5 (2H, t, H-7), 1.4 (2H, m, H-22), 1.3 (1H, q, H-8), 1.2 (2H, m, H-2), 1.19 (3H, s, H-19), 0.89 (2H, m, H-11), 0.86 (6H, m, H-26, H-27), 0.84 (3H, d, H-21), 0.82 (3H, m, H-29), 0.68 (3H, s, H-18); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 36.51 (C-1), 31.31 (C-2), 70.65 (C-3), 41.98 (C-4), 165.53 (C-5), 126.24 (C-6), 202.73 (C-7), 45.58 (C-8), 45.96 (C-9), 38.47 (C-10), 21.38 (C-11), 38.85 (C-12), 43.27 (C-13), 50.07 (C-14), 26.21 (C-15), 29.26 (C-16), 54.85 (C-17), 12.16 (C-18), 17.49 (C-19), 34.10 (C-20), 17.49 (C-21), 36.26 (C-22), 26.51 (C-23), 41.98 (C-24), 28.74 (C-25), 19.20 (C-26), 19.11 (C-27), 23.20 (C-28), 12.16 (C-29).

7-Hydroxysitosterol (III): UV λ_{max} (MeOH) nm: 209.5; IR ν_{max} (KBr) cm^{-1}: 3390 (O-H str), 2944 (CH_3, C-H str), 2876 (CH_2, C-H str), 1662 (=C-H str), 1451 (CH_3, C-H bend), 1371 (=C-H bend); LRMS m/z (rel. int.): 431 ($(M+1)^+$, 4), 415 (4), 414 (25), 413 (100), 400 (1), 399 (7), 395 (7), 380 (3), 212 (3), 175 (3), 159 (4), 145 (6), 143 (6), 135 (7), 119 (7), 107 (7), 95 (8), 91 (8), 81 (9), 79 (7), 69 (7), 69 (7), 57 (12), 55 (14), 43 (17); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 5.24 (1H, s, H-6), 3.48 (1H, t, H-7), 3.21 (1H, m, H-3), 1.94 (2H, m, H-4), 1.66 (1H, m, H-8), 1.59 (1H, m, H-9), 1.51 (1H, m, H-14), 1.46 (1H, m, H-17), 1.33 (1H, d, H-20), 1.30 (1H, m, H-25), 0.62 (3H, s, H-19), 0.54 (6H, m, H-26, H-27), 0.44 (3H, m, H-29), 0.31 (3H, s, H-18). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 37.58 (C-1), 31.55 (C-2), 71.54 (C-3), 42.42 (C-4), 146.44 (C-5), 124.04 (C-6), 65.56 (C-7), 42.17 (C-8), 49.60 (C-9), 37.17 (C-10), 20.88 (C-11), 39.33 (C-12), 42.32 (C-13), 55.87 (C-14), 24.49 (C-15), 28.47 (C-16), 55.85 (C-17), 12.17 (C-18), 18.98 (C-19), 36.29 (C-20), 18.44 (C-21), 34.07 (C-22), 26.04 (C-23), 45.98 (C-24), 29.27 (C-25), 20.00 (C-26), 19.20 (C-27), 23.22 (C-28), 11.82 (C-29).

Antibacterial and antifungal activity studies

Table 1 shows the diameters of the zones of inhibition produced by the root and stem extracts of *G. diversifolia* in comparison with standard antibiotics. The extracts exhibited varying degrees of antibacterial activity against test microorganisms. Overall, ethyl acetate extract exhibited the best antibacterial activity profile. It had the highest activity against *Bacillus pumilus*

and *Escherichia coli*. Petroleum ether extract showed good activity against *Staphylococcus aureus* comparable to benzylpenicillin.

Tested against *C. albicans* and *A. niger*, ethyl acetate extract exhibited higher antifungal activity than any other extract. As shown in Table 1, the diameters of the zones of inhibition produced by the ethyl acetate extract against both fungi were higher than those produced by any other extract.

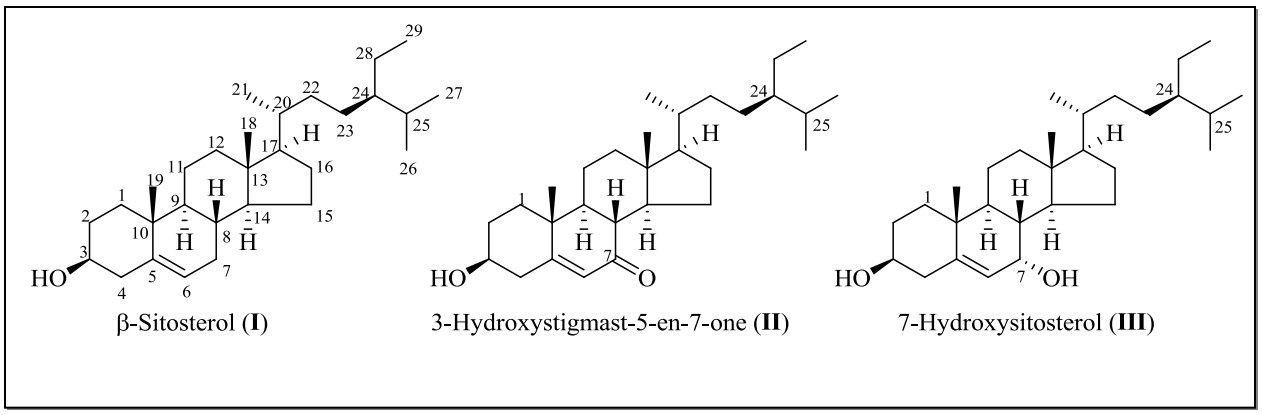

Figure 1. The chemical structures of the isolated compounds.

Table 1: Antimicrobial activity of the root and stem extracts of *Girardinia diversifolia*

Test solution[a]	Diameters of the zones of inhibition[b] (mm)				
	B. pumilus	***E. coli***	***S. aureus***	***C. albicans***	***A. niger***
GdRP	8.1	8.3	8.2	6.3	5.6
GdSP	7.8	8.3	7.9	5.9	5.5
GdRC	7.7	8.0	7.6	5.7	5.7
GdSC	7.4	7.4	7.4	5.8	5.6
GdRM	7.7	7.2	6.6	7.2	6.8
GdSM	7.6	7.1	6.6	7.4	7.5
EAPRM	8.8	8.6	7.2	8.3	8.0
EAPSM	8.8	8.4	7.1	8.9	8.3
Gentamicin	17.0	18.2	-	-	-
Benzylpenicillin	-	-	9.0	-	-
Nystatin	-	-	-	15.6	15.2

[a]Concentrations: extracts 50 mg/ml; gentamicin 10 µg/ml; benzylpenicillin 1 I.U/ml; nystatin 150 µg/ml; [b]mean zone size from duplicate tests; - = not tested; GdRP – *G. diversifolia* root petroleum ether extract; GdSP – *G. diversifolia* stem petroleum ether extract; GdRC – *G. diversifolia* root chloroform extract; GdSC – *G. diversifolia* stem chloroform extract; GdRM – *G. diversifolia* root methanol extract; GdSM – *G. diversifolia* stem methanol extract; EAPRM - ethyl acetate partition of root methanol extract; EAPSM - ethyl acetate partition of stem methanol extract.

Bioautography

Table 2 shows the R_f values of bioactive components. In the ethyl acetate extract, the most active antibacterial components had R_f values of 0.50, 0.44, 0.39, 0.34, 0.29 and 0.24 while the chloroform extract had bioactive components at R_f values 0.53, 0.5, 0.44, 0.39, 0.29 and 0.24. In the antifungal activity studies, one component in the ethyl acetate extract at an R_f value of 0.5 exhibited remarkable activity against *S. cerevisiae*. This component appears to possess both antibacterial and antifungal activities.

Table 2: Retention factors of bioactive components in the root and stem extracts

Extract	Bioactive component (^aR_f)	^{b}Activity score	
		Antibacterial	Antifungal
EAPRM/EAPSM	0.50	+++	++++
	0.44	+++	-
	0.39	++	-
	0.34	+	-
	0.29	+	-
	0.24	+	-
GdRM/GdSM	0.50	+	-
	0.44	+	-
	0.39	+	-
	0.34	+	-
	0.29	+	-
GdRC/GdSC	0.53	+++	-
	0.50	+++	-
	0.44	++	-
	0.15	++	-
GdRP	0.46	+	-

[a]Retention factor as determined in a 90:10% v/v $CHCl_3$:MeOH mobile phase system; [b]Activity score based on the zone of inhibition produced by a bioactive component in comparison to that produced by gentamicin or nystatin standard as appropriate.

CONCLUSION

In preliminary screening for antibacterial and antifungal activities, inhibition diameters higher than 8 mm are generally considered as positive results [7]. The ethyl acetate extract exhibited zones of inhibition with diameters higher than 8 mm against *B. pumilus*, *E. coli*, *C. albicans* and *A. niger*. The petroleum ether extract produced zones of inhibition greater than 8 mm against *S. aureus*. These observations indicate that *G. diversifolia* contain bioactive components with potential antibacterial and antifungal activities.

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