

ANTIFUNGAL ACTIVITY AND ACUTE TOXICITY OF STEM BARK EXTRACTS OF *DRYPETES GOSSWEILERI* S. MOORE-EUPHORBIACEAE FROM CAMEROON

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Abstract

Drypetes gossweileri S. Moore is a plant used in traditional medicine in Cameroon. The antifungal properties of its stem-bark crude extract and fractions DG₁, DG₂, DG₃, DG₄, DG₅, DG₆, DG₇, DG₈ and DG₉ were assayed by agar and broth dilution methods on solid and liquid media against *C. Krusei*, *C. albicans*, *C. glabrata*, *T. mentagerophytes*, *M. langeronii*, *M. gypseum*, *M. audouini*, *T. rubrum*, *T. soudanense*, *T. terrestre*, *A. flavus* and *A. niger*. The results revealed a substantial antifungal effect with minimal inhibitory concentrations ranging respectively from 24.11µg/ml to 1562µg/ml for yeasts and from 3125µg/ml to 12500µg/ml for filamentous fungi. Among the fractions, fraction DG₄ exerted the highest antifungal activity. Moreover, no toxic effect was noticed in male and female albinos Wistar rats treated *per os* with the crude stem bark's extract of *Drypetes gossweileri* at a dose up to 12g/kg of body weight. The phytochemical screening of the crude extract and fractions showed the presence of alkaloids, phenols, flavonoids, saponins, anthocyanines, anthraquinones, sterols, lipids and essential oils. Therefore, *Drypetes gossweileri* may be safe as phytomedicine for the treatment of fungal infections.

Key words: Antifungal activity, *Drypetes gossweileri*, acute toxicity.

Introduction

Numerous studies have recently shown increases in fungal infections and many others established the antifungal potentials of plants (Amvam et al., 1998; Ngono et al., 2000). The advent of synthetic drugs in the health care system coupled with industrialization, in developed countries has made the use of herbal productions as medicine to decline gradually right from the beginning of the 20th century up to the 1970s. In the course of last three decades, there has been renewal and growing interest in the use of plant-derived biologically active compounds as drugs or leads in the pharmaceutical industries (Houghton and Raman, 1998). Today highly effective pharmaceutical drugs are of plant origin.

Drypetes gossweileri is a common Euphorbiaceae in Central Africa, where it is widely used to treat helminthic diseases and rheumatism (Walker and Sillians, 1961). In the Congo, the root of the plant is employed in the treatment of wounds and toothache (Troupin, 1983). Many researches described the antibacterial (Ijah and Oyebanji, 2003), antioxidant and antiradical (Agnaniet et al, 2003) effects of *Drypetes gossweileri*. The previous phytochemical analysis of the extracts indicated the presence of steroids, triterpenoids, alkaloids, saponins with antimicrobicidal properties (Dupont et al, 1997).

The present study aims to evaluate the antifungal properties of the stem bark extracts of *Drypetes gossweileri* and the acute toxicity of its crude extract.

Material and Methods

Plant material

Drypetes gossweileri stem bark were collected at the Eloundem Mountain, and authenticated by Mr Nana victor at the Cameroon National Herbarium, Yaoundé where a vouched specimen was conserved under the identification number 5746/SRF/Cam.

Extraction and fractionation

The plant material was dried at room temperature and ground to powder. The powder (2516 g) was macerated in 8l CH₂Cl₂/MeOH (1:1) for 48h. The filtrate was concentrated to dryness under vacuum to obtain the dark- purple residue. The percent extraction yield was 4.53%. The crude extract (113.97 g) was fractionated by flash chromatography on silica gel (70-230 mesh, 120g) column using an increasing polarity solvent system: Hexane (Hex), Hexane-Ethyl Acetate (Hex-AE), Ethyl Acetate (AE), Ethyl Acetate-Methanol (AE-MeOH), and Methanol (MeOH) gradients (table 1). The afforded fractions were pooled according to their TLC (Thin Layer Chromatography) profile. These extract and fractions were kept at 4°C prior to testing. Before use, they were dissolved in 10% DMSO to give away different concentrations to be used in the test.

Table 1: chromatogram of *Drypetes gossweileri* extracts (25.6g).

Eluent	Fractions	Volume (ml)	Weight (g)	Observations
Hex pure	DG1 (1-5)	500	3	3 visible marks at UV
Hex-AE 75 :25	DG2 (6-13)	700	7	3 marks with 2 visible
Hex-AE 50 :50	DG3 (14-20)	600	4	5 marks with 4 visible
Hex-AE 25 :75	DG4 (21-24)	400	2	4 marks with 3 visible
AE pure	DG5 (25-30)	500	7	Trail with 2 marks visible
AE- MeOH 95 :5	DG6 (31-37)	600	3	6 marks with 1 visible
AE- MeOH 90 : 10	DG7 (38 -45)	700	6	5 marks + trail
AE- MeOH 85 : 15	DG8 (46 -55)	850	13	4 marks + trail
MeOH Pure	DG9 (56-65)	800	5	2 marks + trail

Phytochemical screening

The methods described by Harbone (1976) and Odebeyi and Sofowora (1978) were used to assess the main group of chemical substances (alkaloids, anthraquinones, anthocyanes, flavonoids, phenols, steroids, tannins, triterpenes, saponins, sterols, lipids, reducing sugars, essential oils, coumarins) present in the stem bark crude extract and fractions of *Drypetes gossweileri* and its frations.

**Antifungal assays
Microorganisms**

Eleven pathogenic strains of fungi were used in the study. Three yeasts (*Candida albicans*, *Candida krusei* and *Candida glabrata*), seven dermatophytes (*Microsporium gypseum*, *Microsporium langeronii*, *Microsporium audouini*, *Trychophyton rubrum*, *Trychophyton soudanense*, *Trychophyton mentagrophytes*, *Trychophyton terrestre*) and two moulds (*Aspergillus flavus* and *Aspergillus niger*). These strains were kindly provided by the “Centre Pasteur du Cameroun”. They were maintained in culture on Sabouraud-Glucose (4%) Agar (SGA) medium.

Antifungal screening test

The preliminary antifungal screening on filamentous fungi was done by the food poisoning method (Ngono et al., 2000). The strains were cultured on SGA medium in 55 mm Petri dishes. For each extract or fraction, 100µl were aseptically mixed with 1,9 ml of SGA to final concentrations of 50mg/ml for the crude extract and 25mg/ml for the fractions. 10% DMSO and amphotericine B were used as negative and positive control respectively. After solidification, an explant of 6 mm diameter of a particular dermatophyte or mould was inoculated at the center of the Petri dish and incubated at 25°C for 7 days and 5 days for dermatophytes and moulds respectively. Growth diameters were there after measured and used to calculate the percentage of inhibition.

For yeasts, SGA was poured on 90 cm Petri dishes. After solidification, an inoculum of yeasts strains standardized at 2.5×10^5 CFU/ml on Malassiez cell was spread on the surface of the solid medium. Following the pre-incubation time of 15 mins, wells were hollowed and 100µl of the crude extract (50 mg/ml), fractions (25 mg/ml) and the positive control amphotericine B from Sigma (100µg/ml) were introduced in individual wells respectively. This was done in triplicate. Inhibition zone diameters were measured after 24 hrs of incubation at 37°C.

Minimum inhibitory concentration (MIC)

The MIC which is the concentration of an extract that inhibits any visible growth of the microorganism was determined by the broth microdilution method on yeasts (Ngono et al., 2000). SGB (Sabouraud Glucose 2% Broth) and each extract or fraction was mixed and a serial two-fold dilution was done ranging from 24.41 to 25000µg/ml and 50 µl of a suspension of spores (2.10^4 CFU/ml) was introduced into each well of a 96 wells microtiter plate. The negative control wells received 10% DMSO. Amphotericin B (Sigma) was used as positive control, at concentration ranging from 0.012 to 12.5µg/ml. The microplates were incubated for 48 hrs at 37°C and the MIC determined as the lowest concentration which did not discolored the phenol red used as indicator of yeast growth. For dermatophytes, the food poisoning technique was used as previously described. Each extract concentration ranged from 3125 to 25000µg/ml and the MIC determined as the lowest concentration of the extract that exhibited 100% inhibition.

Minimum fungicidal concentration (MFC)

Incubated microtiter plates (for yeasts) and Petri dishes (for dermatophytes and moulds) were subcultured on free-

extract culture media and incubated at 37 and 25°C respectively and the MFC determined as the lowest concentration at which no growth was observed.

Acute toxicity

The plant crude extract was tested for acute toxicity on male and female albino Wistar rats according to the WHO experimental procedure (1992). Extract doses of 0, 4, 8, and 12g/Kg body weight were administered *per os* to 4 groups of animals of both sex. The rats were observed first 48hrs for death and for 7 days for toxic effects.

Results and Discussion

The preliminary antifungal activities of the extracts are presented in Tables 2 and 3. The fraction DG₅ was found to be the most active fraction against yeasts with an average inhibition zone diameter of 18.66 mm (Table 2). *C. albicans* was the most sensitive among the yeast. In Table 3, we noticed a significant activity of the crude extract and fractions on the dermatophytes and moulds tested. The fraction DG₄ present the highest antidermatophytic activity (80.71%) as shown in Table 3. In addition, *M. gypseum* and *T. mentagrophytes* were the most sensitive dermatophytes whereas *Aspergillus Niger* the less sensitive.

Table 2: Antifungal activity of the crude extract and fractions of *Drypetes gossweileri* against yeasts (Growth inhibition zone in mm).

	DG _b	DG ₁	DG ₂	DG ₃	DG ₄	DG ₅	DG ₆	DG ₇	DG ₈	DG ₉	A
C.A.	20±1.1 ^a	13±1 ^c	15±0.5 ^c	18±0.6 ^b	15±0.6 ^c	16±1 ^b	17±0.7 ^b	13±1 ^c	14±1 ^c	18±1.1 ^b	23±1 ^a
C.K.	0±0 ^e	12±0.7 ^d	10±0.6 ^d	16±1 ^b	10±1.1	20±0.3 ^a	15±0.4 ^c	12±1 ^d	12±1 ^d	14,0±1.2 ^c	22±1 ^a
C.G.	22±1.2 ^a	10±0.4	18±0.7 ^b	17.0±1.2 ^b	21±0.8 ^a	20±0.6 ^a	20±1.3 ^a	0±0 ^e	10±1 ^d	10±0.9 ^d	21±1 ^a

Legend : DG_b : Crude extract, DG₁ : Hex pure fraction, DG₂ : Hex-AE 25% fraction, DG₃ : Hex-AE 50% fraction, DG₄ : Hex-AE 75% fraction, DG₅ : AE pure fraction, DG₆ : AE-MeOH 5% fraction, DG₇ : AE-MeOH 10% fraction, DG₈ : AE-MeOH 15% fraction, DG₉ : MeOH pure fraction, C.A. : *Candida albicans*, C.G. : *Candida glabrata*, C.K. : *Candida krusei*. a, b, c, d and e link values which present no significant difference based on the Student Test (P < 0,05).

Table 3: Antifungal activity of the crude extract and fractions of *Drypetes gossweileri* against dermatophytes and moulds (Percent inhibition).

	DG _b	DG ₁	DG ₂	DG ₃	DG ₄	DG ₅	DG ₆	DG ₇	DG ₈	DG ₉	A
M.L.	100±0 ^a	53.1±1.6 ^h	70.5±1.1 ^f	70±1 ^f	80.2±0.6 ^d	41.6±0.8 ⁱ	70.5±1.6 ^f	45.8±2.2 ⁱ	67.7±0.7 ^f	68.7±0.7 ^f	100±0 ^a
M.G.	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a
M.A.	98.1±1.5 ^a	76.2±0.5 ^e	100±0 ^a	87.3±2.8 ^d	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	95±0 ^b
T.R.	100±0 ^a	84.6±1.3 ^d	87.5±2.1 ^d	98.3±1.1 ^a	100±0 ^a	100±0 ^a	92.8±1.3 ^c	76±1 ^e	67±1 ^f	63.2±0.7 ^e	85±0 ^d
T.S.	96±2 ^b	100±0 ^a	89.3±0.6 ^c	64.1±1.6 ^g	100±0 ^a	97±0 ^b	97.1±1.3 ^b	100±0 ^a	86.3±1.1 ^d	75.2±1 ^e	82±0 ^d
T.M.	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a
T.T.	60.3±1.2 ^g	86.4±3.1 ^d	97.1±1.6 ^b	93.3±1.2 ^c	96.2±2.2 ^b	74.9±2.9 ^e	86.1±1.7 ^d	100±0 ^a	99.1±3.6 ^a	73.7±1.1 ^e	0±0 ^k
A.F.	100±0 ^a	64.9±0.6 ^g	50±2	61.3±0.3 ^g	50±1 ^h	64±2 ^g	58±1.8 ^h	66±1.3 ^f	59±2 ^h	55.1±1.3 ^h	100±0 ^a
A.N.	10±0 ^j	0±0 ^k	0±0 ^k	0±0 ^k	0±0 ^k	0±0 ^k	0±0 ^k	0±0 ^k	0±0 ^k	0±0 ^k	100±0 ^a

Legend: 0-25% = less or no inhibition, 25-50% = average inhibition, 50-75% = good inhibition, 75-100% = very good inhibition. a, b, c, d, e, f, g, h, i, j and k link values which present no significant difference based on the Student Test (P < 0,05). M.L. : *Microsporium langeronii*, M.G. : *Microsporium gypseum*, M.A. : *Microsporium audouini*, T.R. : *Trichophyton rubrum*, T.S. : *Trichophyton soudanense*, T.M. : *Trichophyton mentagrophytes*, T.T. : *Trichophyton terrestre*, A.F. : *Aspergillus flavus*, A.N. : *Aspergillus niger*. DG_b : Crude extract, DG₁ : Hex pure fraction, DG₂ : Hex-AE 25% fraction, DG₃ : Hex-AE 50% fraction, DG₄ : Hex-AE 75% fraction, DG₅ : AE pure fraction, DG₆ : AE-MeOH 5% fraction, DG₇ : AE-MeOH 10% fraction, DG₈ : AE-MeOH 15% fraction, DG₉ : MeOH pure fraction.

The above results prompted the analysis of antifungal effect shown in Table 4. The crude extract and the fractions were active against all tested strains. MIC ranged from 24.41 to 1562.5 µg/ml for the yeast and 3125 to 12500 µg/ml

for dermatophytes and the moulds. *C. krusei* was the most sensitive yeast with MIC ranging from 24.41µg/ml to 97.65µg/ml. The most interesting activity was found with fraction DG₄.

Table 4: Minimal Inhibitory Concentrations (µg/ml) of the crude extract and fractions against selected fungal strains.

	DG _b	DG ₁	DG ₂	DG ₃	DG ₄	DG ₅	DG ₆	DG ₇	DG ₈	DG ₉	A
C.A.	24.41	781.25	195.31	97.65	195.31	195.31	97.65	390.6	781.2	195.31	2.44
C.K.	N.D.	97.65	97.65	24.41	48.84	24.41	24.41	48.84	97.65	48.84	2.44
C.G.	48.84	781.25	781.25	N.D.	195.35	195.31	97.65	195.31	1562.5	781.25	2.44
M.L.	12500	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.44
M.G.	12500	6250	6250	6250	3125	6250	12500	3125	3125	6250	2.44
M.A.	N.D.	12500	6250	N.D.	3125	3125	N.D.	6250	3125	3125	2.44
T.R.	12500	6250	6250	N.D.	6250	6250	6250	N.D.	N.D.	N.D.	2.44
T.S.	N.D.	3125	3125	3125	6250	3125	6250	6250	N.D.	N.D.	2.44
T.M.	6250	3125	3125	3125	3125	3125	3125	3125	3125	3125	3.125
T.T.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	6250	N.D.	N.D.	3.125
A.F.	12500	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3.125

Legend : M.L. : *Microsporium langeroinii*, M.G. : *Microsporium gypseum*, M.A. : *Microsporium audouini*, T.R. : *Trichophyton rubrum*, T.S. : *Trichophyton soudanense*, T.M. : *Trichophyton mentagrophytes*, T.T. : *Trichophyton terrestre*, A.F. : *Aspergillus flavus*, C.A. : *Candida albicans*, C.G. : *Candida glabrata*, C.K. : *Candida krusei*. DG_b : Crude extract, DG₁ : Hex pure fraction, DG₂ : Hex-AE 25% fraction, DG₃ : Hex-AE 50% fraction, DG₄ : Hex-AE 75% fraction, DG₅ : AE pure fraction, DG₆ : AE-MeOH 5% fraction, DG₇ : AE-MeOH 10% fraction, DG₈ : AE-MeOH 15% fraction, DG₉ : MeOH pure fraction, N.D. : Not determined, A : Amphotéricine B.

From the results presented in Tables 5 and 6, overall extracts of *Drypetes gossweileri* showed MFC values ranging from 48.81 to 6250µg/ml for yeasts and 3125 to 25000µg/ml for dermatophytes and moulds, with the majority of fungicidal indices ≤ 4.

Table 5: Minimal Fungicidal Concentrations (µg/ml) of *Drypetes gossweileri* crude extract and fractions on selected fungal strains.

	DG _b	DG ₁	DG ₂	DG ₃	DG ₄	DG ₅	DG ₆	DG ₇	DG ₈	DG ₉	A
C.A.	97.65	12500	390.31	195.31	781.25	781.25	195.31	781.25	6250	390.31	2.44
C.K.	N.D.	195.31	195.31	97.65	97.65	48.81	195.31	390.62	195.31	97.65	2.44
C.G.	195.31	1562.5	1252.5	N.D.	390.3	781.25	390.3	781.25	6250	1562.5	2.44
M.L.	25000	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.44
M.G.	25000	25000	12500	12500	25000	12500	12500	6250	6250	12500	2.44
M.A.	N.D.	25000	12500	N.D.	12500	6250	N.D.	12500	12500	12500	2.44
T.R.	25000	N.D.	6250	6250	25000	12500	6250	N.D.	N.D.	N.D.	2.44
T.S.	N.D.	N.D.	3125	6250	6250	3125	12500	25000	N.D.	N.D.	2.44
T.M.	12500	12500	6250	6250	12500	12500	12500	6250	3125	3125	3.125
T.T.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	12500	N.D.	N.D.	3.125
A.F.	25000	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3.125

Legend : M.L. : *Microsporium langeroinii*, M.G. : *Microsporium gypseum*, M.A. : *Microsporium audouini*, T.R. : *Trichophyton rubrum*, T.S. : *Trichophyton soudanense*, T.M. : *Trichophyton mentagrophytes*, T.T. : *Trichophyton terrestre*, A.F. : *Aspergillus flavus*, C.A. : *Candida albicans*, C.G. : *Candida glabrata*, C.K. : *Candida krusei*. DG_b : Crude extract, DG₁ : Hex pure fraction, DG₂ : Hex-AE 25% fraction, DG₃ : Hex-AE 50% fraction, DG₄ : Hex-AE 75% fraction, DG₅ : AE pure fraction, DG₆ : AE-MeOH 5% fraction, DG₇ : AE-MeOH 10% fraction, DG₈ : AE-MeOH 15% fraction, DG₉ : MeOH pure fraction, N.D. : Not determined. A : Amphotéricine B.

The results presented in Table 7 revealed that the most common constituents found in the extract and fractions are alkaloids, phenols, flavonoids, saponines, anthraquinones, tannins, anthocyanins, sterols, lipids and essential oils. The alkaloids, flavonoids, saponines, tannins, anthraquinones, coumarine, essential oil are known to possess antifungal activities (Bouchet et al., 1986, Cowan, 1999; Bruneton, 1999; Sautour et al., 2004). Anthocyanins, flavonoids, and tannins, have been reported by Barnabas and Nagarajan (1988), and Burapedjo and Bunchoo (1995), to inhibit cell wall formation in fungi leading to the death of the microorganism. This supports the fungicidal activities exerted by the extract and fraction of *Drypetes gossweileri*. The differential distribution of the bioactivity among fractions might be explained by proportions of different bioactive components from each fraction acting either in a synergetic or potentiating ways.

The study of the acute toxicity of the crude extract of *Drypetes gossweileri* stem bark showed no acute toxic effect in rats at doses ≤ 12g/Kg through oral route.

Table 6: MFC/MIC values of *Drypetes gossweileri* crude extract and fractions against selected fungal strains.

	DG _b	DG ₁	DG ₂	DG ₃	DG ₄	DG ₅	DG ₆	DG ₇	DG ₈	DG ₉	A
C.A.	4	N.D.	2	2	4	4	2	2	4	2	1
C.K.	N.D.	2	2	4	2	2	4	4	2	2	1
C.G.	4	2	2	N.D.	2	8	4	2	2	2	1
M.L.	2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1
M.G.	2	4	2	2	8	2	1	2	2	2	1
M.A.	N.D.	2	2	N.D.	4	2	N.D.	2	4	4	1
T.R.	N.D.	N.D.	1	1	4	2	1	N.D.	N.D.	N.D.	1
T.S.	N.D.	N.D.	1	2	2	1	2	4	N.D.	N.D.	1
T.M.	2	4	2	2	4	4	4	2	1	1	1
T.T.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2	N.D.	N.D.	1
A.F.	4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1

Legend : M.L. : *Microsporium langeroinii*, M.G. : *Microsporium gypseum*, M.A. : *Microsporium audouini*, T.R. : *Trichophyton rubrum*, T.S. : *Trichophyton soudanense*, T.M. : *Trichophyton mentagrophytes*, T.T. : *Trichophyton terrestre*, A.F. : *Aspergillus flavus*, C.A. : *Candida albicans*, C.G. : *Candida glabrata*, C.K. : *Candida krusei*. DG_b : Crude extract, DG₁ : Hex 100% fraction, DG₂ : Hex-AE 25% fraction, DG₃ : Hex-AE 50% fraction, DG₄ : Hex-AE 75% fraction, DG₅ : AE 100% fraction, DG₆ : AE-MeOH 5% fraction, DG₇ : AE-MeOH 10% fraction, DG₈ : AE-MeOH 15% fraction, DG₉ : MeOH 100% fraction, N.D. : Not determined. A : Amphotéricine B.

Table 7: Phytochemical analysis of the crude extract and fractions of *Drypetes gossweileri*.

Extracts	DG _b	DG ₁	DG ₂	DG ₃	DG ₄	DG ₅	DG ₆	DG ₇	DG ₈	DG ₉
Al	+	+	-	-	+	+	+	+	-	+
Po	+	+	+	+	+	+	+	+	+	+
Fl	+	+	+	+	+	-	+	-	+	+
Tr	-	-	-	-	-	-	-	-	-	-
Sa	+	-	-	-	-	-	-	+	+	+
An	+	-	+	+	+	+	+	+	+	+
Ta	+	-	-	-	+	+	-	+	-	-
Co	-	-	-	-	-	-	-	-	-	-
Aq	+	-	-	-	-	+	+	+	+	+
He	+	+	+	-	-	-	-	-	-	-
St	+	+	-	+	-	-	-	-	-	-
Li	+	+	+	+	-	-	-	-	-	-

Legend : Al : Alkaloids, Fl : Flavonoids, Po : Phenols, St : Steroids, Ta : Tanins, He : essential oils, An : Anthocyanes, Co : Coumarins, Tr : Triterpenes, Aq : Anthraquinones, Sa :Saponins, Li : Lipid, (+) : present, (-) : absent, DG_b : Crude extract, DG₁ : Hex pure fraction, DG₂ : Hex-AE 25% fraction, DG₃ : Hex-AE 50% fraction, DG₄ : Hex-AE 75% fraction, DG₅ : AE pure fraction, DG₆ : AE-MeOH 5% fraction, DG₇ : AE-MeOH 10% fraction, DG₈ : AE-MeOH 15% fraction, DG₉ : MeOH pure fraction.

Conclusion

Results achieved in this study, in addition to the lack of toxicity observed in rats support further investigation of extracts of *Drypetes gossweileri* stem bark as potential antifungal agents.

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References

1. Agnanié, A., Mounzeo, H., Menut, C., Bessière, J.M., Criton, M. (2003). The essential oils of *Rinorea subintegriifolia* O. ktze and *Drypetes gossweileri* S. Moore occurring in Gabon. Flavour and fragrance journal. 18 (3): 207-210..
2. Amvam Zollo, P.H, Biyiti, L., Tchoumboungang, F., Menut, C., Lamaty, G. and Bouchet, Ph. (1998). Aromatic plants of tropical central Africa. Part XXX II Chemical composition and antifungal activity of thirteen essentials oils from aromatic plants of Cameroon. Flavour and fragrances Journal. 13: 107-114.
3. Barnabas, C.G., Nagarajan, S.(1988). Antimicrobial activity flavonoids of some medicinal plants. Fitoterapia. 3: 508-10.
4. Bouchet, Ph., Masanes, C. and Delaude, C. (1986). Activités antidermatophytes et anti-levures d’une série de saponines extraites de végétaux récoltés au Zaïre. Bulletin de la société Française de Mycologie Médicale. 15 (2): 533-538.
5. Bruneton, J. (1999). Pharmacognosie, Phytochimie, Plantes médicinales. 3è édition revue et augmenté. Ed. TEC & DOC Paris. 1120p.

6. Burapedjo, S, Bunchoo, A. (1995). Antimicrobial activity of tannins from *Terminalia citrina*. *Planta Medica*. 61: 365–6.
7. Cowan, M.M. (1999). Plants Products as Antimicrobial Agents. *Clinical Microbiology Review*. 12(4):564-582.
8. Dupont, M.P., Liabrés, G., Delauche, C., Tchissambou, L. and Gastman, J.P. (1997). Sterolic and triterpenoidic constituents of stem bark of *Drypetes gossweileri*. *Planta Medica*. 63: 282 – 284.
9. Harbone, J.B. (1976). *Phytochemicals Methods. A guide to modern technique of plant analysis*. Edition Chapman and Hall, London. Pp 1-150.
10. Houghton, P.J., Raman, A. (1998). *Laboratory handbook for fractionation of natural extracts*. Chapman and Hall, London. 199p.
11. Ijah, U.J.J., Oyebanji, F.O. (2003). Effects of tannins and polyphenols of some medicinal plants on bacterial agent of urinary tract infections. *Global Journal of Pure and Applied Sciences*. 9 (2): 193 – 198.
12. Ngono, N. A., Biyiti L., Amvam Zollo, P.H and Bouchet, Ph. (2000). Evaluation of antifungal activity of extracts of two Cameroonian Rutaceae: *Zanthoxylum leprieurii* Guill. & Perr. *Zanthoxylum xanthoxyloides* waterm. *Journal of Ethnopharmacology*. 70: 335 -342.
13. Odebeyi. O.O. and Sofowora, F.H. (1978). Antimicrobial alkaloids from a Nigerian Chewing Stick (*Fagara zanthoxyloides*). *Planta Medica*, 36: 204-207.
14. Sautour, M., Mitaine-O, .A.C., Miyamoto, T., Dongmo, A. and Lacaille-D, .M.A. (2004). Antifungal steroid saponines from *Dioscorea cayenensis*. *Planta Medica*. 70(1) : 90-92.
15. Troupin G. (1983). *Flore des plantes ligneuses du Rwanda*. Musée royal de l’Afrique Centrale Turuven. Pp257 – 258.
16. Walker, A.R. and Sillians, R. (1961). *Les plantes utiles du Gabon*. Tournon, 6è Ed, Paris, Pp. 165-167.
17. WHO (1992). *Research guidelines for evaluating the safety and efficacy of Herbal medicine*. WHO, Regional Office for Western Pacific, Manila, Philippines, 38p.