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*Short Communication*

# *In vitro* assay of potential antifungal and antibacterial activities of extracts of *Borassus aethiopum Mart*

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ABSTRACT: The anti-inflammatory, antipyretic, and pro-apoptotic properties of extracts of *Borassus aethiopum* have been reported in the literature. In this study, we investigated the antifungal and antibacterial properties of *Borassus aethiopum* male inflorescences extracts. The antifungal and antibacterial activity was studied by agar well diffusion method *in vitro*. The effect of antibacterial potential was examined against Gram positive bacteria (*Staphylococcus aureus*), and Gram negative bacteria i.e., (*Escherichia coli* and *Enterobacter cloacae*). In the antifungal activity assays, the dermatophytes strains *Trichophyton rubrum, Trichophyton interdigitale, Trichophyton soudanense, Microsporum langeronii,* and *Epidermophyton floccosum* were used. The E2F2 extract showed strong inhibitory activity on four of the five fungal species used against ketoconazole, a standard antifungal drug. However, the E2F2 extract displayed weak antibacterial activity against the bacterial strains tested. The results of the present study support the ethnomedicinal uses of *Borassus aethiopum* for the treatment of fungal diseases. The phytochemical screening of E2F2 extract revealed the presence of sterols, triterpenes and saponins, witch may be involved in the antifungal activity.

KEYWORDS: *Antifungal, antibacterial, Borassus aethiopum*



*Borassus aethiopum Mart* (Arecaceae) is a tropical plant found widely spread in Africa. This plant is used in alimentation (Glew et al., 2005), in technology (Nethaji et al., 2010) and in traditional medicine. In traditional medicine *Borassus aethiopum* is used for multiple purposes. All parts (roots, leaves, flowers, fruits) of the plant are used in traditional medicine (Cassou et al., 1997). Combination of powdered *Borassus aethiopum* male inflorescences with shea butter is used as a cutaneous lesion antifungal remedy. Male inflorescences have been claimed to have diuretic properties, and are also used in the treatment of sexual transmitted diseases (e.g. herpes), and viral infections such as measles (Cassou et al., 1997). Previous studies reported anti-inflammatory, antipyretic, and pro-apoptotic activities of extracts of this plant (Sakande et al 2004a, 2004b, 2011). The present study was aimed at investigating the basis for the traditional use of *Borassus aethiopum* male inflorescences in the treatment infectious diseases. This would be a preliminary step that could justify the search for the active substance in the extracts.

The *Borassus aethiopum* male inflorescences were harvested locally in Ougadogou. Extraction solvents (dichloromethane, methanol) and the phytochemical screening reagents were obtained from Prolabo, France. Antibiotic and antifungal discs were obtained from Biomerieux and were supplied at the following concentrations: amoxicillin (25 µg), oxacillin (5µg), tetracyline (30 µg), erythromycin (15µg), nalidixic acid (30µg), and cefotaxim (30µg), and ketoconazole (50µg).

Clinical dermatophytic fungal strains from the University hospital Yalgado Ouedraogo of Ouagadougou were tested. The fungal strains were *Trichophyton rubrum*, *Trichophyton interdigitale*, *Trichophyton soudanense*, *Microsporum langeronii*, and *Epidermophyton floccosum*. Clinical bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae*) from University hospital Yalgado Ouedraogo of Ouagadougou were tested. The growth media used were obtained from the following sources: Saboureau-Chloramphenicol-Actidione (SAC) and Müller Hinton medium were obtained from Biomerieux (France).

*Borassus aethiopum* male inflorescences were collected and identified by Professor Sita Guinko (Institute of Natural Products Research of Ouagadougou, Vegetal Biology and Ecology Laboratory of UFR SVT). A voucher specimen AA1522 was deposited at the Herbarium. The inflorescences were air-dried in the shade and

powdered. The powder was exhaustively extracted by percolation with 3 litres of dichloromethane-methanol (50:50). The fraction obtained (E2F2 extract) was evaporated under reduced pressure to obtain 10 g of residue. Phytochemical screening was carried out according to the methodology for chemical analysis for vegetable drugs (Ciulei, 1982).

**TABLE 1 Chemical screening of *Borassus aethiopum* extracts.**

| Substances | $E_2F_2$ | Reactions |
|---|---|---|
| Sterols and Triterpenes | +++ | Liberman-Buchard |
| Flavonosides | - | Shibata |
| Saponins | ++ | Hemolysis |
| Alkaloids | - | Dragendorf and Mayer |
| Anthracenosides | - | Bornträger |

Antifungal activity was evaluated using the agar well diffusion method (Shathele 2010). This was achieved by uniformly spreading 1 ml of fungal suspension prepared with sterile 0.85% physiological saline solution on Saboureaud Chloramphenicol Actidione (SAC) plates. After inoculum absorption by SAC, wells were made using sterile cock borers, which were then filled with 0.1 ml of the different concentrations of E2F2 extract dissolved in tween 80 (5 mg/ml). The control was carried out by filling the wells with 0.1 ml of tween 80 dissolved in sterile distilled water. The same procedure was followed for the determination of the diameter of inhibition (DI) of the antifungal agent Ketoconazole. Plates were incubated in steam room at 27 °C. The results were read 5 days later. Standard diameter of inhibition was 25 ± 5. Every test was carried out in triplicate. For antibacterial activity determination, all the strains were cultured overnight at 37 °C in Müller-Hinton broth. Discs of amoxicillin (25 µg), oxacillin (25 µg), tetracyline (30 µg), erythromycin (15 µg), nalidixic acid (30 µg), cefotaxim (30 µg) and E2F2 extracts (12.5, 25, 50, 100 mg/ml) were tested. The antimicrobial activities were determined by measuring the growth inhibitory zones using the agar-well diffusion method (Kronvall, 2011). The results were read 24 hours later. Every test was carried out in triplicate. Data were expressed as Mean ± SEM of triplicates. Student's t-test was used to compare antibacterial and antifungal activities of the extracts against the standard antifungal and antibacterial agents. All statistical analysis was conducted with SPSS software (V. 12, SPSS, USA) at significant level of 0.05.

The results of phytochemical screening (presented in Table1) showed the presence of sterols, triterpenes and saponins in E2F2. The diameters of inhibition of E2F2 extract are reported in Table 2. The DI values obtained at the concentrations of E2F2 tested (12.5 - 100 mg/ml) showed that the strains were sensitive with the exception of *Trichophyton soudanense*. The DI obtained for E2F2 (100 mg/ml) on *Trichophyton rubrum* and *Microsporum langeronii* are superior to the DI of the ketoconazole used as a reference drug in this investigation. This study showed that four of the dermatophytes studied; *Trichophyton rubrum*, *Trichophyton interdigitale*, *Microsporum langeronii* and *Epidermophyton floccosum* were very sensitive to extract E2F2. *Trichophyton soudanense* was resistant to this extract, even at the highest concentration tested (100 mg/ml).

Results presented in Table 3 reveal weak antibacterial activity of E2F2 extract (diameter of inhibition 12 and 11 mm) on *Escherichia coli* and *Enterobacter cloacae* respectively) at a dose of 100 mg/ml. The extracts were found to be completely ineffective at lower concentrations (12.5 - 50 mg/ml). The E2F2 extract was totally ineffective against the *Staphylococcus aureus* strain. Our investigation on the potential antifungal activities of *Borassus aethiopum* male inflorescences in this study showed that four dermatophytic fungi (*Trichophyton rubrum*, Trichophyton interdigitale, *Microsporum langeronii* and *Epidermophyton floccosum*) were sensitive to the E2F2 extract. The DI of E2F2 (100 mg/ml) on *Trichophyton rubrum* and *Microsporum langeronii* was superior to the DI of ketoconazole, a standard antifungal drug used as a reference in our experiments. This observed antifungal activity of E2F2 is of significant medical and economic importance in Burkina Faso where the high cost of pharmaceutical antifungal drugs affects the quality of healthcare. In fact, mycoses such as ringworm are very frequent especially among the poor populations. Recently, the number of infections caused by these fungi has increased considerably causing particular concern, especially when immuno-compromised patients are infected. Particularly, in HIV-infected patients, the symptoms are typical and the lesions are more severe and extensive (Gupta et al. 2001; Shathele, 2010).

**TABLE 2 Diameters of inhibition (DI in mm) of E2F2 extract on five dermatophytes (standard DI of Ketoconasole = 25 ± 5 mm).**

| | E2F2[1] | | | | Ketoconasol (50 µg) |
|---|---|---|---|---|---|
| | 12.5 | 25 | 50 | 100 | |
| *Trichophyton rubrum* | 28.4 ± 0.8 | 31.5 ± 1.0 | 36.1 ± 2.1 | 40.7 ± 1.2 | 39±1.0 |
| *Trichophyton interdigitale* | 18.3 ± 0.5 | 22.4 ± 0.4 | 27.1 ± 0.8 | 30.6 ± 2.2 | 40 ± 0.5 |
| *Microsporum langeronii* | 29.5 ± 0.4 | 33.6 ± 0.9 | 37.8 ± 1.1 | 41.7 ± 1.9 | 40 ± 1.0 |
| *Epidermophyton floccosum* | 24.5 ± 0.3 | 27.3 ± 0.7 | 32.7 ± 0.5 | 36.2 ± 0.7 | 41 ± 0.6 |
| *Trichophyton soudanense* | 0 | 0 | 0 | 0 | 38±0.4 |

[1]Sample concentration (mg/mL)
Sensitive compare to standard DI

The findings of this study lend weight to the ethnomedicinal uses of *Borassus aethiopum* for the treatment of fungal diseases. These dermatophytes utilize keratin as a source of nutrient causing different skin infections referred to as tineas in man or ringworm in man and animals. The phytochemical screening of E2F2 revealed the presence of sterols, triterpenes and saponins compounds responsible of the antifungal activity. Indeed these compounds are well known for their antifungal activities (Nasimul-Islam et al 2003, Abdulmoniem et al, 2006, Hassan et al 2007, Sunday et al 2009, Chowdhury et al 2011). Contrary to the antifungal activity, the extract E2F2 showed a weak antibacterial activity against multiple-drug-resistant bacteria. The DI was respectively 12 mm and 11 mm against *Escherichia coli* and *Enterobacter cloacae* respectively when applied at a dose of 100 mg/ml. The diameter of inhibition of extract E2F2 on *Enterobacter cloacae* (11mm) was close to that of cefotaxim (DI: 10 mm), a third generation cephalosporin.

**TABLE 3 Diameters of inhibition (DI in mm) of E2F2 extract on bacteria.**

| | Standard DI (mm) | *E. coli* | *E. cloacae* | *S. aureus* |
|---|---|---|---|---|
| Amoxicilline (25 µg) | 24.5 ± 2.25 | 0 | 0 | 9 ± 0.5 |
| Oxacilline (5 µg) | 30.5 ± 3.25 | 0 | 0 | 14 ± 0.7 |
| Erythromycin (15 µg) | 29 ± 2.5 | 13.3 ± 1 | 0 | 23 ± 0.5 |
| Cefotaxime (30 µg) | 35 ± 2.5 | 30 ± 0.9 | 10 ± 0.5 | 26 ± 0.4 |
| Nalidixic acid (30 µg) | 28 ± 2.5 | 25 ± 0.7 | 0 | 13 ± 0.6 |
| Tetracycline (30 µg) | 18 ± 1.0 | 0 | 0 | 8 ± 1 |
| E2F2 (12.5 mg/ml) | - | 0 | 0 | 0 |
| E2F2 (25 mg/ml) | - | 0 | 0 | 0 |
| E2F2 (50 mg/ml) | - | 0 | 0 | 0 |
| E2F2 (100 mg/ml) | - | 12 ± 0.4 | 11 ± 0.6 | 0 |

Note that the hospital strain of *Enterobacter cloacae* was resistant to amoxicillin, oxacillin, erythromycin, acid nalidixic and to tetracycline. We also obtained a diameter of inhibition of 12 mm for *Escherichia coli* which was otherwise resistant to amoxicillin, oxacillin and tetracycline. The diameter of inhibition of E2F2 for Escherichia coli was close to that of erythromycin. This inhibitory activity was observed only at the high dose of 100 mg/ml). Furthermore, it is noteworthy that the activities obtained were rather weak (11 and 12 mm), and unlikely to have meaningful effect when used for a clinical application. However these strains were multi-resistant drug strains. It would be relevant to perform this test using reference strains, which would allow us to better measure the intensity of the antibacterial activity of E2F2.

The findings from this study provide a scientific basis for the ethnomedicinal uses of *Borassus aethiopum* in the treatment of fungal diseases. The extract used here need to be characterised and standardised in order for it to be useable for the management of the frequent incidence of mycoses in Africa.

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