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Antifungal and antioxidant activity of crude extracts of three medicinal plants from Cameroon pharmacopea

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Three medicinal plant (Millettia laurentii, Tephrosia vogelli, Croton macrostachyus) extracts traditionally used in Cameroon to manage infectious diseases, were chosen and screened for their phytochemicals composition, antioxidant properties and antifungal activity against Trichophyton rubrum, Trichophyton soudanense and Trichophyton violaceum. Plant powders were extracted by maceration in methanol and water. Afforded extracts were studied for their phytochemical composition using indicators, antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging methods and antifungal activity using the agar dilution methods. Extraction yield were better with methanol than water for all the medicinal plants. Amongst the 11 phytochemical compounds (alkaloids, phenols, flavonoids, triterpenoids, saponines, anthraquinones, tannins, anthocyanins, coumarins, essential oils, steroids, glycosides, lipids) tested, 9 were differently present in relative high amount in extracts, while glycosides and steroids were absent in all plant extracts. Aqueous extracts of T. vogelli showed the best antioxidant activity while methanolic extract of C. macrostachyus showed the lowest with IC50 values of 0.30 and 0.11 mg/ml, respectively. M. laurentii showed IC50 of 0.19 and 0.21 mg/ml for aqueous and methanolic extracts, respectively. All the extracts tested showed significant activity against the three Trichophyton species tested, with minimum inhibitory concentration (MIC) and minimum fungicidal concentrations (MFC) varying from 17.50 to 27.50 and 20 to 30 mg/ml, respectively. These findings support the continued sustainable screen of medicinal plants as source of bioactive principles.

Key words: Medicinal plant, phytochemical screening, antifungal, antioxidant.

INTRODUCTION

Fungal diseases are classified at the fourth range of nosocomial infections (Carle, 2003; Bouguerra et al., 2004; Barchiesi et al., 2006). Victims of fungi infections can be found elsewhere in the world, with the predomination in a third of the world’s countries (43%) (World Health Organization, 1998). In Cameroon, mycosis prevalence was 40.61% and is probably grown up right now (Mungyeh, 1999). Since few decays, considerable increase of these infections has been noted, mainly due to increase risk factors such as human immune deficiency virus (HIV), tuberculosis, malnutrition and the development of resistance to pre-existing antifungal drugs coupled to their high cost (Ghannoum and Rice, 1999; Sanglard and Odds, 2002). The development of resistance to drug by pathogens and toxic side effects of available antifungal therapies have emphasized the search for new efficient and none or less-toxic antifungal drugs (Bouguerra et al., 2004; Barchiesi et al., 2006; Thiel, 2007; Azor et al., 2007; Nisle et al., 2008; Perlin, 2009).

Medicinal plant is the oldest way used by humans to cure diseases because of their therapeutic value (Nostro...
et al., 2000; OMS, 2002). The recorded use of plants in the treatment of ailments dates back to antiquity (Sofowora, 1993). Research on bioactive substances from plant sources has great scope and could lead to the development of new antifungal agents able to combat fungal resistance (Jung, 1976; Facheux et al., 2003). Various herbs and spices have been reported to exhibit antioxidant activity, mostly due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins (Aqil et al., 2006). Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases (Devasagayam et al., 2004).

Traditionally, *Milletia laurentii* a Fabaceae plant families is used as antitumoral, anti-inflammatory, antiviral, bactericidal, insecticidal and pest-destroying. These activities have been confirmed through pharmacological studies thereby thwarting the interest of these genera in traditional medicine as source of bioactive compounds. *Tephrosia vogelli* hook F. is a perennial small leguminous shrub with mauve flowers and many pods. Its well known properties are ichthyotoxic, insecticidal, food parasiticidal, antifeedant, antibacterial and pest crops control (Ibrahim et al., 2000; Wanga et al., 2000). *Croton macrostachyus* is a deciduous tree 3 to 25 m high, with large spreading branches, common in secondary forests between 200 to 2000 m of altitude. Plants of this genus are known for their antidiabetic, vasorelaxant, antimalarial, antiulcer and anticonvulsive activities (Ngadjui et al., 2002; Baccelli et al., 2007; Okokon and Nwafor, 2009). The petroleum ether extract of *Croton zambesicus* leaves has an antifungal activity (Abo et al., 1999), and the alkaloidal fraction of leaves’ ethanolic extract inhibits *Aspergillus* and *Microsporum* species (Block et al., 2004).

Therefore, this work aimed to evaluate the antifungal activities and the antioxidant properties of three Cameroonian plants named *M. laurentii*, *T. vogelli*, and *C. macrostachyus* through the study of their phytochemical screening, antioxidant properties, and their action against three fungal strains.

**MATERIALS AND METHODS**

**Plant samples**

Leaves of *M. laurentii* collected in the campus of Yaoundé I University in April, 2010, *T. vogelli* and *C. macrostachyus* collected in the same month in year 2010 at Bamelang in the west region of Cameroon were also used in this study. All samples were authenticated at the National Herbarium of Cameroon (Yaoundé) where specimens were deposited under the reference numbers: N°1487/SRF/CAM for *M. laurentii*, N°10356/SRF/CAM for *C. macrostachyus* and N°24450/SRF/CAM for *T. vogelli*.

**Fungi strains**

*Trichophyton rubrum*, *Trichophyton soudanense* and *Trichophyton violaceum* isolate obtained from the “Centre Pasteur du Cameroun” were used for antifungal activities of plant extracts.

**Plant extraction**

The dried leaves of *M. laurentii*, *T. vogelli* and *C. macrostachyus* were ground into fine powder using an electric blender. 500 g of each powder was soaked in 1.8 L of distilled water and 1.8 L of methanol, respectively for 72 h. Thereafter, the mixture was filtered through Whatman paper.

The water extract was dried in an oven at 45°C for 3 consecutive days. To accelerate the evaporation of water, the extracts were first put inside trays before introduced in the oven until the complete drying. Methanolic extract were concentrated using a rotary evaporator (BUCHI 011) at 65°C. The obtained water and methanolic extracts were further used for photochemical screening, antioxidant and antifungal as follows.

**Phytochemical screening**

The extracts were subjected to qualitative phytochemical screening for the presence of flavonoids according to Harborne (1976), alkaloids, triterpenoids, saponines, anthraquinones, tannins, steroids, glycosides according to Evans (2002), phenols, anthocyanins, coumarins, essential oils and lipids according to Odeybei and Sofowora (1978).

**Antioxidant activity of plant extracts**

The antioxidant activity of each aqueous and methanolic plant extracts was measured in terms of radical scavenging ability, according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Katalinié et al., 2003). DPPH free radical (violet color) was reduced by antioxidant. The stronger the antioxidant present in the plant extract, the fainter the solution color was. 20 µl of extract was introduced into 2 ml of a methanolic solution of DPPH (0.3 mM) and kept in the dark for 30 min. Methanol was used as the negative control, and catechin was used as the standard. The absorbance was read spectrophotometrically at 517 nm, using the SHIMADZU UV Visible 2000 apparatus. The antioxidant content and inhibition rates of DPPH radical were calculated as milligram of catechin equivalent per gram of plant. IC50 values (in mg/ml) expressed the concentration of samples necessary to scavenge 50% of DPPH free radicals. IC50 values were calculated by Probit analysis (Finney, 1980).

**Antifungal activity assay**

Agar dilution method was used for minimum inhibitory concentration (MIC) and minimum fungicidal concentrations (MFC) determination (Berghoe and Vlietnick, 1991). Plant extracts were serially two-fold diluted with drug-free agar medium (SDA), resulting in final concentrations ranging from 10 to 30 mg/ml. Explants of 7 mm diameter were seeded in triplicate at the centre of the Petri dish that was sealed and incubated at 37°C for seven days. Drug-free SDA was prepared, inoculated and incubated in the same condition as negative control. Amphotericin B (SIGMA) at 2.44 µg/ml was used as positive control. The growth diameters were subsequently measured. The lowest concentration at which no growth was observed was considered as the MIC. Petri dish in which no fungal growth was observed was sub-cultured on drug-free agar medium (SDA). The dishes were incubated at 37°C for 48 h. The lowest concentration exerting complete visible growth inhibition was considered as the MFC.
Table 1. Extraction yield of *Croton macrostachyus*, *Tephrosia vogelli*, and *Milletia laurentii*.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. vogelli</em> (a)</td>
<td>10.38</td>
</tr>
<tr>
<td><em>T. vogelli</em> (m)</td>
<td>12.4</td>
</tr>
<tr>
<td><em>C. macrostachyus</em> (a)</td>
<td>3.76</td>
</tr>
<tr>
<td><em>C. macrostachyus</em> (m)</td>
<td>5.66</td>
</tr>
<tr>
<td><em>M. laurentii</em> (a)</td>
<td>5.85</td>
</tr>
<tr>
<td><em>M. laurentii</em> (m)</td>
<td>9.65</td>
</tr>
</tbody>
</table>

*T. vogelli* (a): Aqueous extract of *T. vogelli*, *T. vogelli* (m): methanol extract of *T. vogelli*, *C. macrostachyus* (a): aqueous extract of *C. macrostachyus*, *C. macrostachyus* (m): methanol extract of *C. macrostachyus*, *M. laurentii* (a): aqueous extract of *M. laurentii*, *M. laurentii* (m): methanol extract of *M. laurentii*.

Statistical analyses

The results are presented as means ± standard deviation (SD). Data were analyzed using the statistical package for social sciences (SPSS) 10.1 software for Windows. The mean values were compared using Student-Newman-Keuls test with *p* < 0.05.

RESULTS

Extraction yields

The powdered *T. vogelli*, *C. macrostachyus* and *M. laurentii* produced, respectively 3.76 to 10.38% for aqueous extracts and 5.66 to 12.4% for methanolic extracts (Table 1).

Phytochemical screening results

The extracts (methanol and aqueous) of the three plants contained saponin, phenolic compound, tannins, anthocyanins, steroids, triterpenes, alkaloids, coumarins, anthraquinones, glucosides and essential oils. Glucosides and steroids were absent in the methanol and aqueous extracts of the three plants. Anthocyanins were present only in the methanol extracts of *T. vogelli*. Saponin and triterpenes were absent in the methanol and aqueous extract of *C. macrostachyus* (Table 2).

Antioxidant activity results

Among the six extracts tested and the standard for the *in vitro* antioxidant activity using the DPPH method, the methanolic extracts of *C. macrostachyus* and the aqueous extracts of *T. vogelli* showed the lowest and the highest antioxidant activity, with IC$_{50}$ values of 0.30 and 0.11 mg/ml, respectively (Figure 1). Significant difference was recorded for the antioxidant activities between aqueous and methanolic extracts of *C. macrostachyus* and *T. vogelli*, however solvent did not influence the antioxidant activity of *M. laurentii* because the recorded values are practically the same.

Antifungal activity results

All extracts showed significant antifungal activity against the tested fungi (Table 3). The MIC and MFC varied from the fungi isolates with respect to the type of plant extract ranging from 17.5 to 27.5 and 20 to 30 mg/ml, respectively. Generally, the crude extracts of our samples exhibit less MIC and MFC than the standard antifungi amphotericin B (Table 3).

DISCUSSION

Yield extraction of the three plants greatly varies according to the plant species, the part of plant used (steams, roots, leaves, fruits) and solvent type used for the extraction (Table 1). Such variation could be related to edaphic factors as well as intrinsic genetic of each plant species (Bohm, 1987; Nishanta and Bohm, 1999). The yield extraction variation of the same plant species with respect to the solvent could be explained by the differential solubility of chemical compounds in different solvent. A number of studies related to the extraction of chemicals compounds from plant with different solvent found that there is high variation of the yield (Ennajar et al., 2009).

Plant spices generally produce natural antioxidants responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Those antioxidants have free radical scavenger’s properties and generally include flavonoids, coumarins, anthocyanins, tannins, essential oils and phenolic compounds (Ennajar et al., 2009; Bettaieb et al., 2010). Our study showed the presence of these compounds in the aqueous and methanolic extracts of our plants essentially (Table 2). However, the nature of those compounds greatly varies according to the solvent types. In general, the methanolic extract of all the plant tested have more phenolic compounds than the aqueous extracts (Table 2). Previous study of Datsu et al., (2009) on *Croton zambesicus* identified glycosides, flavonoids, and terpenes in the ethyl acetate extract while those of Okokon and Nwafor (2010) in roots ethanolic extract of the same plant identified saponines, alkaloids, terpenes, cardiac glycosides, anthraquinones. Makoshi and Arowolo (2011) indicated the presence of saponins, cardiac glucosides and flavonoids in the leaves extract of *T. vogelli*. In the present paper, we have evaluated the free radical scavenger activity of methanolic and aqueous extract of *M. laurentii*, *T. vogelli* and *C. macrostachyus*. These plants are traditionally used by the local population
Table 2. Phytochemical compounds present in the crude aqueous and methanol extracts of *C. macrostachyus*, *T. vogelli*, *M. laurentii*.

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>T. vogelli</em> (a)</th>
<th><em>T. vogelli</em> (m)</th>
<th><em>C. macrostachyus</em> (a)</th>
<th><em>C. macrostachyus</em> (m)</th>
<th><em>M. laurentii</em> (a)</th>
<th><em>M. laurentii</em> (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Phenolic</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpens</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Coumarins</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Antra-quinins</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Essential oils</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>


Table 3. Minimal inhibition and fungicidal concentrations (mg/ml) of the crude extracts of *T. vogelli*, *C. macrostachyus*, and *M. laurentii*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>T. vogelli</em> (a)</th>
<th><em>T. vogelli</em> (m)</th>
<th><em>C. macrostachyus</em> (a)</th>
<th><em>C. macrostachyus</em> (m)</th>
<th><em>M. laurentii</em> (a)</th>
<th><em>M. laurentii</em> (m)</th>
<th>Standard Amphotericin B (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (mg/ml)</td>
<td>27.5</td>
<td>27.5</td>
<td>27.5</td>
<td>17.5</td>
<td>20</td>
<td>20</td>
<td>2.4</td>
</tr>
<tr>
<td>MFC (mg/ml)</td>
<td>30</td>
<td>30</td>
<td>20</td>
<td>17.5</td>
<td>20</td>
<td>20</td>
<td>2.4</td>
</tr>
</tbody>
</table>

MIC: Minimal inhibitory concentrations, MFC: Minimal fungicidal concentrations. *Tv(a)*: Aqueous extract of *T. vogelli*, *Tv(m)*: methanol extract of *T. vogelli*, *Cm(a)*: aqueous extract of *C. macrostachyus*, *Cm(m)*: methanol extract of *C. macrostachyus*, *Ml(a)*: aqueous extract of *M. laurentii*, *Ml(m)*: methanol extract of *M. laurentii*. *Tr*: Trichophyton rubrum, *Ts*: Trichophyton soudanense, *Tv*: Trichophyton violaceum. ND: Not determined.

To solve their problem of health (Wagate, 2008). Among the six extracts and standard tested for their in vitro antioxidant activity using the DPPH method, the crude methanolic extracts of the three plants always show better activity compared to the aqueous extract (Figure 1). This observation was previously shown with many plant extracts, generally attributed to a range of photochemical compounds like polyphenols, flavonoids, tannins, anthocyanins (Lee et al., 2004; Ennajar et al., 2009; Bettaieb et al., 2010). It is obvious that the constituents like tannins, essential oils present in the extract may be responsible for such activity. However, the chemical constituents present in the extract, potentially responsible for this activity, need further investigation.

The results obtained on the antifungal activity of *M. laurentii*, *T. vogelli* and *C. macrostachyus* showed that these plant extracts possess antifungal properties and can be effective antibiotics since they inhibited the growth of fungal causative agents of skin diseases (Table 3). This observation is in line with the work of Ajaiyeoba et al. (1998). The plant extracts might be host specific
in their antifungal activity since MIC varied from one fungal strain to another. The different rates of inhibition may probably be due to the quantity of the phytochemical compounds present in each extracts. However, M. laurentii did not go in line with this observation, as the MIC of both extracts have the same value within the experiment. This could be explained by the relative low amount or the absence of many group of compound in the extracts of this plant compared to others.

It is known that tannin and flavonoids have antimicrobial activities. It is also known that at low concentration, tannins can inhibit the growth of microorganisms and act as an antifungal agent at higher concentration by coagulating the protoplasm of the microorganism (Onadapo and Owonubi, 1993). The bioactive constituents, anthocyanins, flavonoids, phenolic compounds and tannins may have played major roles in the activity of our extracts against Tricophyton fungi strains, as the presence of these compounds in other plants have been reported with their potential to inhibit the cell wall formation of fungi leading to the death of the organism (Barnabas and Nagarajan, 1998; Burapedjo and Bunchoo, 1995).

This current experiment therefore provides some scientific justification for the utilization of extracts from these plants by the local population to treat skin disease. Although the potency of our extracts were below that of amphotericin B, bio-guided fractionation may bring out active principle with higher or equal potency than that of the standard molecules.

**Conclusion**

The present study supports the continued sustainable use of medicinal plant resources for health purposes and highlight scientific interest to this field. This will enable a wide use of these plant locally or elsewhere in the world. Phytochemical exploration of the principles of these plants should be undertaken to isolate the active biological principles that may lead to the development of novel antifungal agents.

**REFERENCES**


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