Phytochemical analysis and antimicrobial activity of *Boerhaviacoccinea*

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**ABSTRACT**

This study was aimed to evaluate the phytochemical profile and antimicrobial activity of the leaf and root methanolic extracts of *Boerhaviacoccinea* P.Mill, of the family Nyctaginaceae. Phytochemical analysis confirmed the presence of tannins and flavonoids and absence saponins in both root and leaves extracts. Whereas, terpenenes were detected in root extract and not in the leaves and glycosides were detected in leaves extract but not in the root extract. The in vitro antimicrobial testing was carried by using six pathogenic microbes that included; four bacteria (Bacillus anthracis (NCTC10073), Streptococcus pyogens (clinical isolate), Vibrio cholera (clinical isolate), Shigelladyentriae(clinical isolate)) and two fungi; Candida albicans (ATCC90028) and Cryptococcus neoformans (clinical isolate). Moderate inhibitory activity with MIC value of 0.625 mg/mL against Cryptococcus neoformans and MIC value of 1.250 mg/mL against Candida albicans were obtained from leaf and root extracts respectively. Both extracts exhibited weak activity of 5.00 mg/mL against Bacillus anthracis whereas, Streptococcus pyogens, Vibrio cholerae, Shigelladyentriae were resistant at the maximum concentration tested (MIC > 5.00 mg/mL). In the light of our results, phytochemicals with antifungal activity are present in both leaves and roots. The traditional use this plant has been validated. The activity against Cryptococcus neoformans provides is an additional data that was not indicated by information providers.

**Keywords:** *Boerhaviacoccinea;* Antibacterial; Antifungal; Phytochemical analysis

**INTRODUCTION**

Since ancient times, plants have been used to treat various human and animal ailments. They are rich in natural chemicals and the main sources for pharmaceuticals, nutraceuticals, cosmetics and food supplements (Surivayanthana et al., 2012). Infectious diseases are the leading cause of morbidity and mortality especially in the developing countries (WHO, 2004). The clinical efficiency of many existing antimicrobial drugs is threatened by the emergence of multi-drug resistant pathogens which pose a continuous need to discover new antimicrobial compounds with diverse chemical structures and possibly novel mechanisms of action for new and re-emerging infectious diseases (Rojas et al., 2003). Biomolecules of plant origin appear to be one of the alternatives, used by many societies, for the control of various antibiotic resistant human pathogens (Raghavendra et al., 2006).

In developing countries especially in the tropical areas rich in plants, herbs play a key role in treatment of disease. People continue using plants that are effective. Thus, the probability of discovering novel therapeutic agents are higher when plant selection is based ethnomedicinal information from reliable sources.

*Boerhaviacoccinea*, P. Mill, is commonly known as Scarlet Spiderling or Red Spiderling plant, belongs to the family Nyctaginaceae. It is a stout perennial, prostrate to decumbent, multi-stemmed, many-branched herb having a diffusely paniculate presentation of flowers, with stems up to 1.5ft. (3 - 50 cm) long, radiating outward forming a mat of herbaceous growth (Halvorson and Guertin, 2003).

Although some sources consider plantname synonymous with Boerhaviadiffusa, reputable databases like Integrated Taxonomic Information Systems (ITIS) and the w3 TROPICOS (Missouri Botanical Garden) identifies them as different taxa (Halvorson and Guertin, 2003). Genetical studies based on the chromosome count confirmed that, *Boerhaviacoccinea* and Boerhaviadiffusa are two different species (Spellenberg, 2000).

In Tanzania the leaves of *Boerhaviacoccinea* are used against oral candidiasis and aphthous ulcers. (Maregesi et al., 2007). The methanolic extract exhibited antiplasmodial activity of 250 µg/ml and antiHIV activity against HIV1 IIIB strain with IC50 values of 54.85 and 37µg/ml for methanolic and water extract respectively.

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(Maregesi et al., 2010). Recently the plant leaves were reported for use against fungal infection of the scalp. Also, leaves are used as vegetable by some tribes of Tanzania e.g. Rangi and Luo (Maregesi, Personal Communication, 2012).

To the best of our understanding, phytochemical data is lacking, and the biological testing is scanty compared to the related species such as B. diffusa of which various pharmacological studies such as antimicrobial against various pathogenic microbes, anti-inflammatory, antioxidant, antihepatopatic activity etc. has been established and even a phytomedicine patented (Oladele et al., 2011; Khalid et al., 2011; Muthulingum, 2008; WO2009044040 (A1), 2009).

**MATERIAL AND METHODS**

**Chemical reagents and media**

Dimethylsulphoxide was purchased from Sigma (Poole, Dorset, England), Fluconazole was purchased from CA-DILA pharmaceutical Ltd (Dholka India), Gentamicin susceptibility test discs (10μg) were purchased from Oxoid (Oxoid, Basingstoke, Hampshire, England). Iodonitrotetrazolium chloride was purchased from SIGMA Aldrich, St Louis, USA. Tryptone soya agar and Tryptone soya broth were purchased from Himedia Laboratory Pvt Ltd (Mumbai, India). Sabouraud dextrose agar and broth were obtained from Biotec Laboratory Ltd (Ipswich, United Kingdom). Reagents and solvents used were of analytical grade purchased from the Lab Equip Ltd. Dar Es Salaam, Tanzania.

**Plant material collection authentication**

Leaves and roots were collected from the Botanical Garden located at Muhimbili University of Health and Allied Sciences, Dar Es Salaam Tanzania. Authentication was done at the herbarium unit, Department of Botany, University of Dar Es Salaam.

**Plant material processing and extracts preparation**

The collected roots were freed from soil, cut into small pieces, dried in shade and powdered using a blender. Fresh leaves were pounded using mortar and pestle to increase the surface area.

60 gm of the dry powdered root was extracted by maceration using 98% Methanol (3 x 500mL) at room temperature with occasional shaking. The micella were pooled together and filtered through 22 μm filter paper followed by drying using a vacuum rotor evaporator at about 40°C and freeze drier to yield 4.76 gm (7.4%) dry extracts.

680 gm of pounded leaves was extracted three times by maceration by using 98% Methanol (1200 mL for 72 hours, 2 x 750 mL for 48 hours) with occasional shaking. The micella were pooled together filtered through 22 μm filter paper dried as above to yield 18.0 gm (2.65%) dry extracts.

**Qualitative analysis of phytochemicals**

Chemical tests were carried out on extracts using standard procedures to identify the constituents as described by Trease and Evans (2004) and Mushli et al. (2012).

**Antimicrobial testing**

**Tested microorganisms**

The extracts were tested against authentic pure cultures of human pathogenic vis bacteria namely; Bacillus anthracis (NCTC 10073), Streptococcus pyogens (clinical isolate), Vibrio cholera (clinical isolate) and Shigellaflexneri (clinical isolate), two fungi Candida albicans (ATCC 90028) and Cryptococcus neoformans (clinical isolate). All were obtained from the Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences.

Both antibacterial and antifungal testing used a Broth dilution method following the protocols described by Mwangomo et al. (2012). In this method, a series of dilution of test samples were mixed with a suitable medium, which has previously been inoculated with the test microorganism.

**Antibacterial testing**

MIC was determined by the broth microdilution technique using sterile flat bottomed 96 well polystyrene microtiter plates. Bacterial suspensions equivalent to 0.5 McFarland concentrations were prepared by suspending microbes’ inocula in sterile distilled water and adjusting to get the right turbidity (equivalent to 0.5...
McFarland concentrations). Test solution were prepared by dissolving 20 mg of the extracts into 0.1 ml of DMSO and diluted with 0.9 ml of distilled water to make a concentration of 20 mg/ml. The stock solution (50 µl) was pipetted and added into the first well of each row of plates pre-loaded with 50 µl of tryptone soya broth. Then serial dilution was performed by transferring the test sample from first row wells to wells of the next rows, down to the last rows. The 50 µl from the last row wells were discarded. This was followed by addition of 50 µl of solution containing the test organisms (0.5 McFarland dilutions) to each of the wells. Wells in two columns were used as growth controls, where no drugs were added, while other two were used as the positive controls in which gentamycin was used. The microtitre plates were incubated at 37oC for 24 hours.

After the incubation period 20µl of a 0.2% p-iodonitrotetrazolium chloride (INT) were added to the wells followed by incubation at 37oC for 0.5 h. Presence of microbial growth was indicated by change of INT colour to pink, while absence of growth was indicated by absence of colour change. The lowest concentration at which there was no growth was taken as the minimum inhibitory concentration (MIC).

Antifungal testing

Dilution method was used to determine the antifungal activity of the plant extracts against Candida albicans and Cryptococcus neoformans. Sabourauds Dextrose Agar was used for the preparation of fungal cultures at 37oC for 24 h (agar preparation was done according to the manufacturer’s instructions).

Testing against Candida albicans and Cryptococcus neoformans followed the same procedure as the antibacterial testing described previous with saborauds dextrose broth used instead of tryptone soya broth. Fluconazole was used as a standard positive control.

RESULTS AND DISCUSSION

Phytochemical analysis

The preliminary qualitative analysis of methanol extracts of B. coccinea indicated the presence of tannins, flavonoids, terpenoids and glycoside while saponins could not be detected and can be explained by the fact that, the solvent used for extraction i.e. 98% methanol was not polar enough extract them (see Table 1).

Antimicrobial activity

The selection of the plant, B. coccinea for this study was based on its use to treat fungal infections; the oral thrush and scalp infection in Kiswahili known as ‘Utandu’ and Mashilingi respectively. Infections caused by Candida sp. and C. neoformans are of public health concerns especially that, they most common opportunistic infections among the immuno-compromised individuals includeophoryngeal candidiasis and cryptococcal meningitis (Pfaller and Diekema, 2004). Thus, C. neoformans was included in this study. Both root and leaf extracts of Boerhavia coccinea were found to possess antifungal activity as shown in table 2.

Based on the antifungal assessment suggested by Algiannis et al. (2001), the obtained results are classified as follows; the leaf extract is described as having moderate inhibitory activity against Cryptococcus neoformans with a MIC value of 0.625 mg/mL and weak inhibitory activity with MIC value of 2.50 mg/mL against Candida albicans. The root extract also observed to possess weak inhibitory activity against C. neoformans (MIC value of 2.50 mg/mL) and moderate inhibitory activity MIC of 1.25 mg/mL against Candida albicans. Anticandidal activity is in agreement with the previous report where the n-hexane extract of shoot showed antifungal activity against Candida albicans (Maregesi et al. 2008). Traditionally, water macerate of the shoot is gurgled and drunk for oral thrush while pounded fresh leaves are smeared for the scalp infection and the efficacy is claimed by users. Our observation may in part be influenced by the method used to prepare the extract and test method employed.

Antifungal activity had been reported from previous studies of related species including; Boerhavia diffusa against Aspergillusflavus, Aspergillusniger, and C. albicans (Baskaran et al., 2011) the dermatophytes; Microsporumgypseum, Microsporumfulvum and Microsporumnomas by decreasing the sporulation of these fungal species (Agrawal et al., 2003) and Boerhaviaeucta against Aspergillusflavus, and Aspergillusniger (Suriyavathana et al., 2012).

Both extracts showed very weak antibacterial activity with the MIC value of 5.00 mg/mL against Bacillus anthracis but Streptococcus pyogenes, Vibrio Cholerae and Shigellaflexneri were resistant. Lack of activity of

**Table 2: Antibacterial and antifungal activity**

<table>
<thead>
<tr>
<th>Extract/Reference Drug</th>
<th>Minimum Inhibitory Concentration (MIC) mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ba</td>
</tr>
<tr>
<td>Root extract</td>
<td>5.00</td>
</tr>
<tr>
<td>Leaves extract</td>
<td>5.00</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.00625</td>
</tr>
</tbody>
</table>

Abbreviations: Ba = Bacillus anthracis, Sp = Streptococcus pyogenes, Vc = Vibrio Cholerae, Sf = Shigellaflexneri, Ca = Candida albicans, Cp = Cryptococcus neoformans, ** = MIC is above 5.000 mg/mL, * = MIC is above 5.00 mg/mL
the shoot extracts against Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumonia had been reported for B. coccinea (Maregesi et al. 2008). These results are contrary to previous studies on related species that exhibited antibacterial activity, e.g. Boerhaviaerecta against Aeromonashydrophilia, Bacillus subtilis, and Pseudomonas aeruginosasa (Suriyavathana et al., 2012) and Boerhaviadiffusa against Staphylococcus aureus, Bacillus cereus, and Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosasa, and Klebsiella pneumonia. (Baskaran et al., 2011).

The protein called INDIN-SAA was isolated from the root extract of B. diffusa. This compound exhibited antimicrobial, antioxidative and antilymphoproliferative activity against Scrofula adenitis (tuberculosis of the neck, or, a cervical tuberculous lymphadenopathy), caused by Mycobacterium scrofulaceum. (Khan et al., 2011). Also, the B. diffusa extract is used in the treatment of different skin conditions such as urticaria, eczema and other skin inflammatory conditions. The use of a purified B. diffusa extract that is rich in hydroxybenzoic compounds, such as hydroxybenzoic acid, is an active ingredient in the preparation of a cosmetic composition for skin relief and for increasing skin comfort (WO2009044040 (A1), 2009).

CONCLUSION

Boerhaviacoccinea is a common weed growing in various parts of Tanzania and resistance to diseases. It can easily be cultivated for production of drugs when efficacy and safety are established. Comprehensive scientific study is recommended to facilitate its incorporation in the medicinal and nutritional application. The obtained results support to use of the plant in traditional medicine. Regarding the Cryptococcusneofor-
mans infections, the inhibition (MIC value = 625 µg/mL) of the crude leaf extract is interesting and promising for isolation of bioactive compounds or partial purification to identify the active fraction that could be standardized and formulated when safety is confirmed.

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