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Antimicrobial activity of the seed crude extracts of *Triumfettarhomboidea* (*Tiliaceae*)

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ABSTRACT

Hexane, dichloromethane and methanol extracts of the seeds of *Triumfettarhomboidea*were assayed for their *in-vitro* antimicrobial properties against four bacterial species:*S. typhimurium, E. coli, B. subtilis, B. cereus*and one yeast species- *C. albicans,* using agar disk-diffusion method. All the three extracts exhibited varying degrees of antimicrobial activities, with the dichloromethane extract showing activity against all the microbes tested. This study shows that *T. rhomboidea,* which is currently used as traditionalmedicine in certain parts of the world,is a potential source of antimicrobial compounds.

KEYWORDS: *Triumfettarhomboidea,*antimicrobial activity, medicinal plant.

INTRODUCTION

Triumfettarhomboidea(also known as the Chinese burr) is a perennial herb widely distributed widely across the tropical regions of the world (Royal Botanic Gardens & Domain Trust, 2010)and been used in folk medicine and therapy for the treatment of various diseases, such as gonorrhoea, diarrohea, and leprosy. Various Parts of the plant used therapeutically are fruit, flower, leaves, bark and root. Roots are used as diuretics and in the treatment of intestinal ulcer and the stem barks are used in diarrheoa and dysentry treatment and also as an aphrodisiac. It leaves, flowers and fruit concoctionsact as astringents (Chopra et. al, 1986, Charttegee, 1992, Mukharjee, 2002, Baernes, 2002, Khare, 2007).

Infections caused by bacteria and fungi account for approximately one-half of all deaths in the developing countries. The emergence of multi-resistant microbial pathogens towards the current antimicrobial drugs and the rise in new infectious diseases still remain a serious global threat to human health (WHO, 2008). Developing countries have a rich biodiversity of plant resources that exhibit a wide range of biological activities which may help in the development potent anti-microbial agents. Phytochemicals derived from plants have shown great promise in the treatment of many infectious diseases (Nascimento et al., 2000; Rios and Recio, 2005). These reasons justify the search for more potentially effective and new antimicrobial compounds among the many plant species (Pretorius et al., 2003, Moreillion et al., 2005).

There are about seventyknown species of *Triumfetta* which are distributed. *Triumfettarhomboidea,* is still largely used ethno-medically in various parts of the globe as traditional medicine. The purpose of this study was to investigate the antimicrobial activities of seed extracts of *T. rhomboidea*against selected microbes.

MATERIALS AND METHODS

Plant material extraction procedure

The seeds of *T. rhomboidea*plantwere collected from Rongo District in Western Kenya in August 2008, were dried to constant weight.The taxonomic identification of plant materials was confirmed by Department of Botany, Egerton University, Kenya and where a voucher specimen was deposited at the herbarium section. The collected plant seeds were air dried in the shade for 24 h and pulverized using a milling machine (MILWAUKE WISCONSIN 53207 Model No. 311).

The powdered material (400 g) was first soaked 1 L of hexane for 24 hrs, and then filtered using (Whatman filter paper No. 1) to obtain the hexane extract. The above procedure was repeated in successively with the plant residue to obtain the dichloromethane and methanol extracts. The three crude filtrates were concentrated *in-vacuo*using a Rotary evaporator (BUCHI Rotavapor Model No. R-205) while maintaining the bath temperature at 40°C in order to avoid thermal decomposition of the labile compounds. The crude extracts obtained were then stored

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in pre-sterilized sample vials at –4°C awaiting antimicrobial tests.

Microorganisms

A total of five microorganisms, one yeast species, and five bacterial species, were used as the test organisms in this study. The list of microorganisms used is given in Table 1. Pure cultures of thetest microorganisms were obtained from the Dairy and Food science Technology (DAFTEC) Department of Egerton University, Kenya.

Antimicrobial activity

The stored crude extracts were dissolved in their respective solvents (hexane, dichloromethane and methanol) to a final concentration of 30 mg/ml using 0.45 µm milipore filters. Antimicrobial tests were then carried out by disk diffusion method (Murray et al., 1995) using 100 µl of suspension containing 108 CFU/ml of bacteria, 106 CFU/ml of yeast spread on nutrient agar (NA) and sabouraud dextrose agar (SDA) respectively. The nutrient agar was prepared by weighing 28 g of agar in 1000 ml distilled water in a Erlenmeyer flask and then sterilized for 15 minutes at 121°C in an autoclave before being left to cool. After cooling the media was poured into the Petri dishes ready for the introduction of the microorganism. The disks (6 mm in diameter) were impregnated with 10 µl of the extracts (400 µg/disk) at the concentration of 30 mg/ml and placed on the inoculated agar. Negative controls were prepared using the sterilized paper disks without extracts or antibiotics. Ciprofloxacin was used as a standard. 20 % v/v WFI in DMSO was used as a positive control and clotrimazole (30 µg/mL) was used for fungi. The inoculated plates were incubated at 37°C for 24 h for bacterial strains, and 48 h for yeast isolates after which the test plates were examined. Following incubation, the zones of inhibition formed were measured (in mm) and the mean diameter obtained to assess their respective antimicrobial activities. Each assay in this experiment was done in triplicate. Overall, cultured bacteria with halos equal to or greater than 7 mm and fungi with 10 mm halos were considered susceptible to the tested extract (Okerulu, 2001).

RESULTS AND DISCUSSION

The antimicrobial activities of *Triumfettarhomboidea*, (hexane, dichloromethane and methanol) extracts against the microorganisms (*Candida albicans, Bacillus cereus, Salmonella typhimurium, Escherichia coli, Bacillussubtilis*) were examined in the study. Themicrobe's sensitivity, were qualitatively assessed by the presence or absence of inhibition zones, and the inhibition zone (IZ) diameters. The antimicrobial activity results containing the determined IZ values for the each test organism are reported in table 1.

The results indicate that the dichloromethane extract of *T. rhomboidea* showed activity against all the microbes tested. The hexane extract did not show activity against *C. albicans*, this indicates that the anti-fungal compounds from the seeds are relatively non-polar. *Escherichia coli* showed no sensitivity towards both the hexane extract and the methanolic extracts. *S. typhimurium*was most sensitive to the dichloromethane extract (20.3 mm) while *C. albicans* was least sensitive towards the methanolic extract (8.1 mm).

Table 1. Antimicrobial activity of crude extracts of *Triumfettarhomboidea*

Inhibition zone diameter (mm) around test disk				
	Triumfettarhomboidea **extracts (400 µg/disk)**			
Test Organisms	Hexane	Dichloromethane	Methanol	Positive Control (STD*)
C. albicans		10.1±0.3	11.1±1.1	37.8±0.6
B. cereus	10.2±0.8	12.4±0.4	9.2±0.2	36.6±0.9
S. typhimurium	14.3±0.7	20.3±0.5	15.1±0.7	35.6±0.5
E. coli		8.9±0.3		35.6±0.5
B. subtilis	12.4±0.6	11.4±1.2	10.2±0.5	37.1±0.8

*STD - Positive control for bacterial pathogens was ciprofloxacin and for fungi, clotrimazole.
N/B: All the negative controls showed no activity against the pathogens.

Two gram positive bacterial strains (*B. cereus, and B. subtilis*) were sensitive to hexane, dichloromethane and methanol crude extracts. The gram negative (*E. coli*) was only sensitive to the dichloromethane extract while the gram negative (*S. typhimurium*) was sensitive to the hexane, dichloromethane and methanol extracts. This result suggest that the seed *T. rhomboidea*contains compounds sensitive to both gram positive and gram negative bacteria.

The compounds responsible for the anti-microbial activity in the crude extracts from the seeds of *T. rhomboidea*have varied polarities between polar, mid-polar and very polar. This is because hexane is the least polar of the three solvents and methanol, the most polar. Dichloromethane is considered mid-polar.

CONCLUSIONS

The results of this study suggest that *Triumfettarhomboidea* seeds possess compounds with antimicrobial activity against fungal and bacterial pathogens. Its observed antimicrobial activity provides an explanation for its use in traditional folk medicine. Considering that the plant has beneficial

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antimicrobial activities, it can be cultivated for use as a potential source of antifungal and anti-antibacterial compounds.

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