

ANTI-BACTERIAL ACTIVITIES AND PHYTOCHEMICAL SCREENING OF EXTRACTS OF DIFFERENT PARTS OF *THALICTRUM RHYNCOCARPUM*

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Abstract

Parts of the plant *Thalictrum rhyncocarpum* are used in herbal medicine in Kenya to treat various infections. The aim of this study was to evaluate *in-vitro* anti-bacteria activities and phytochemical profiles of solvent extracts of the leaves, stem bark and root of *Thalictrum rhyncocarpum* against *Bacillus subtilis*-6633, *Staphylococcus aureus*-SG 511, *Escherichia coli* SG 458, *Pseudomonas aeruginosa*-K799/61 and *Mycobacterium vaccae*-10670. Anti-bacterial activity tests were carried out using disc diffusion assay and tube dilution technique, and phytochemical screening was carried out through Thin Layer Chromatography. The crude extracts showed antibacterial effects on *M. vaccae*, *P. aeruginosa* and *B. subtilis*. *M. vaccae* was most sensitive, particularly to the methanol root extract. Phytochemical screening of the extracts suggested the presence of glycosides and alkaloids in the stem bark and root extracts, and flavonoids and triterpenes in the leaf extracts. The study showed interesting levels of activities of solvent extracts of different parts of *T. rhyncocarpum* against some of the bacteria tested (*M. vaccae*, *P. aeruginosa* and *B. subtilis*). The results provide some scientific rationale for the traditional use of the plant in Kenya to treat different microbial infections.

Key words: *Thalictrum rhyncocarpum*, Ethno-medicinal plant, Anti-microbial activities, Phytochemical profile

Introduction

The use of plants in traditional medicine has been well documented in many parts of the world (UNESCO, 1997-1998 and Otieno et al., 2008). According to the World Health Organisation (WHO, 2008), 65% of the world's population have incorporated ethnomedicine in their primary health care practice. In some African and Asian countries, 80% of the population depends on traditional medicine for primary health care and about 70% of population in the developed world has used alternative or complementary medicines (WHO, 2008). In India, traditional healers use about 2500 plant species as a regular source of medicine to treat different diseases (Mathu et al., 2006). However, only a small proportion of medicinal plants (10%) has been studied scientifically (Lai & Roy, 2004; Tapsell et al., 2006). For example, of the plants used to treat microbial infections, an estimated 6% have been screened for specific anti-microbial activities and only a small proportion of these have been studied phytochemically to identify the active constituents and/or blends (Fabrican and Farnsworth, 2001; Tapsell et al., 2006).

The genus *Thalictrum* comprises 120 species of perennial herbaceous plants. In India, species of the genus have been used as tonic, aperient, diuretic, antiseptic and for the treatment of stomach discomfort, snake bites, jaundice, rheumatism and microbial infections (Zerrin et al., 2004). In China, 43 species of this genus are used to treat lung cancer, cough and dysentery (Bao et al., 2003). In West Africa, the plant *T. rhyncocarpum* is used to accelerate wound healing and cure breast cancer (Boily & Puyvelde, 2002). In South Africa, water extracts of leaf, stem bark and roots of *T. simplex* are used to cure viral influenza. In the Democratic Republic of Congo, crude extracts of *T. rhyncocarpum* are used to control *Bacillus subtilis*, *Candida albicans*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Salmonella gallinarum* and *Staphylococcus aureus* (Boily & Puyvelde, 2002).

A number of species in this genus have been screened for *in vitro* anti-bacterial activities against both Gram-positive and Gram-negative bacteria associated with human diseases (Ferzaneh et al., 2008). For example, methanol extract of aerial parts of *T. minus* demonstrated high *in-vitro* activity against *Salmonella typhi* (Ferzaneh et al., 2008). Water extracts of *T. orientale* exhibited anti-microbial activity against *Staphylococcus aureus*, *Salmonella gallinarum*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Candida albicans* at a concentration of 1000 µl/ml (Erdemgil et al., 2004). *T. simplex* methanol and water extracts were found to exhibit *in-vitro* activity against the influenza virus (Julia & Maria, 2003).

Phytochemical studies of the species studied to date have led to the isolation of a large number bioactive alkaloids and some sesquiterpenoids. Almost all the alkaloids belong to the isoquinoline group. Their biological activities include hypotensive, antimicrobial and antitumour properties (Adnan et al., 1999). A novel oxobenzylisoquinoline alkaloid, thalprzewalskiinone, isolated from the roots of *T. przewalskii*, demonstrated *in-vitro* activity against both Gram-positive and Gram-negative bacteria (Adnan et al., 1999). Thalicoside A1, A2, and A3 (structural derivatives of thalprzewalskiinone)

have been isolated from aerial parts of *T. minus* with thalicoside A2 exhibiting *in-vitro* inhibition of the fungus *Candida albicans* and antibacterial activity against *Staphylococcus aureus* (Alexandra et al., 2000). A glycosyl triterpenoid, 3-O- β -D-glucopyranosyl-(1,4)- β -D-fucopyranosyl (22S, 24Z)-cycloart-24-ene-3 β , 22, 26-trio 26-O- β -D-glucopyranoside, isolated from *T. fortunei*, demonstrated anti-tumour activity against NCIH-460 and sac-7901 cancer cells (Xiantao et al., 2011).

In Kenya, *T. rhynchocarpum* has been used extensively in traditional medicine to treat stomach ulcers, snake bites, dysentery and skin rashes (Jeruto et al., 2011). This study reports on *in-vitro* anti-microbial activities of organic extracts of leaves, stem bark and root extracts of *T. rhynchocarpum* and their phytochemical profiles.

Materials and Methods

Plant materials

The leaves, roots and stem barks of *Thalictrum rhynchocarpum* were collected in November 2010 in Ngong forest in Nairobi county, Kenya (37 °08 E and 0 ° 13 S). The plant species was authenticated at the University of Nairobi Herbarium, where a specimen is preserved (Voucher number TR-NG-203).

Plant materials preparation

The collected plant materials were transported to Kenyatta University. The leaves, stem barks, and roots were separated, and these were allowed to dry under shade for a period of 14 days. The air dried plant materials were separately ground into powder using an electric grinder. The powdered materials were then stored at room temperature (21-26 °C) until extracted.

Extraction

Extraction of the plant materials was carried out for three successive days. Ground leaves were extracted with different analytical grade solvents (Fluka) to target different groups of constituents: leaf powder with dichloromethane, stem bark powder using 1:1 mixture of dichloromethane and methanol, and powdered root with methanol (Xiantao et al., 2011). The solvents extracts were filtered separately and concentrated *in vacuo*.

Disc diffusion assay for anti-bacterial activity

The disc diffusion method (Jorgensen et al., 1999; Joseph et al., 2006) was used to screen for antibacterial activities of the crude extracts against the five bacterial strains. Approximately 9 mL of Müller-Hinton agar (Oxoid, UK) was poured into petri dishes (9 cm in diameter) and inoculated with the respective test organisms. Wells (4 mm) were punched out of the solid agar using pipette tips, and 1 ml of 50 μ g/mL of the test extracts or control antibiotic Ciprofloxacin (5 μ g/ml) placed in different wells. The petri dishes were incubated at 30 °C for 20 h and the average diameter of the inhibition zones surrounding the wells computed from three sets of replicates.

Tube dilution assay test

The minimum inhibitory concentrations (MIC) of the extracts were determined using a serial micro-plate dilution assay (Ravi et al., 2010) against each test bacterial species. This was determined by 2-fold serial dilution of the extracts beyond the level where no inhibition of growth of the bacterial strains was observed. Each extract was reconstituted to 100 μ g/ml in DMSO and 100 μ l aliquot which was serially diluted with equal amounts of water in 96-well microplates. The test bacteria were inoculated into Muller-Hinton (MH) broth culture (1%), incubated at 37°C overnight, and 100 μ l aliquots of the resulting culture added to each well. Ciprofloxacin at a concentration of 100 μ g/ml was used as a positive control and untreated wells used as negative controls. The microplates were sealed and incubated at 37 °C and 100% relative humidity for 18 h. As an indicator of bacterial growth, 40 μ L aliquots of a 0.2 mg/mL solution of *p*-iodonitrotetrazolium violet (INT) dissolved in water was added to the microplate wells and incubated at 37 °C for 30 min. The MIC value was recorded as the lowest concentration of the extract at which bacterial growth was inhibited.

Phytochemical screening

The crude extracts of *T. rhynchocarpum* were screened for the presence of terpenes, flavonoids, glycosides and alkaloids using standard qualitative procedures (Jorge et al., 2011). *P*-Anisaldehyde and Dragendorff solutions were used as thin layer chromatography spray reagents.

Results and discussion

Anti-microbial activities of the crude extracts

Table 1 summarises the results of disc diffusion assays of the crude solvent extracts of *T. rhynchocarpum*. All the three extracts showed high activities against *M. vaccae*, moderate activities against *B. subtilis* and *P. aeruginosa*, but no significant activity against *S. aureus* and *E. coli*. The inhibitory activity of methanol root extract against *M. vaccae* was 6.9 ± 1.7 μ g/ml which was significantly higher ($P < 0.05$) than that of the standard synthetic anti-bacteria agent ciprofloxacin

which activity was 10.3 ± 1.3 $\mu\text{g/ml}$. The activity of the leaf extracts against *M. vaccae* was 17.2 ± 1.2 $\mu\text{g/ml}$ while that of stem bark extracts was 15.1 ± 1.4 $\mu\text{g/ml}$, both of which were significantly lower than that of ciprofloxacin.

Table 1: Anti-bacteria activities (as reflected in MIC values) of *T. rhyncocarpum* extracts

Extract code	<i>B. subtilis</i> ($\mu\text{g/ml}$)	<i>S. aureus</i> ($\mu\text{g/ml}$)	<i>E. coli</i> ($\mu\text{g/ml}$)	<i>P. aeruginosa</i> ($\mu\text{g/ml}$)	<i>M. vaccae</i> ($\mu\text{g/ml}$)
1	$31.2^a \pm 2.1$	$>100^a$	$>100^a$	$28.4^b \pm 2.3$	$17.2^a \pm 1.2$
2	$24.4^b \pm 1.3$	$>100^a$	$>100^a$	$42.1^a \pm 2.0$	$15.1^b \pm 1.4$
3	$21.5^c \pm 2.4$	$>100^a$	$>100^a$	$23.6^c \pm 2.2$	$6.9^d \pm 1.7$
Ciprofloxacin	$9.6^d \pm 1.1$	$11.3^b \pm 1.4$	$8.4^b \pm 1.9$	$12.4^d \pm 3.2$	$10.3^c \pm 1.3$

1: Leaf extract; 2: stem bark extract; 3: root extract. Means with different letters in a column are significantly different (SNK, $p \leq 0.05$)

Phytochemical screening of crude extracts

Table 2 summarises results of phytochemical screening of the two extracts. Phytochemical screening of the three extracts suggested the presence of triterpenes, flavonoids and glycosides in leaf, stem bark and root extracts. Since triterpenes and glycosides give similar responses to anisaldehyde, the extracts may contain either of the two classes of compounds or both. The presence of alkaloids was suggested in stem bark and root extracts. Previously, it was reported that the genus *Thalictrum* is rich mainly in benzylisoquinoline alkaloids (Zhu & Xiao, 1991). However, recent reports indicate that the genus is also characterised by several classes of compounds originating from different biosynthetic pathways, including cycloartene-type triterpenes, triterpenoid glycosides (Hitoshi *et al.*, 2000; Ziantao *et al.*, 2011). The genus may also be a unique source of structurally important fatty acids with a *trans*-double bond at C5 (Jinn & Amitabha, 1980). Bio-activity-guided fractionation, isolation and characterisation of the active constituents and blends of *T. rhyncocarpum* extracts are underway.

Table 2: Phytochemical screening of crude extracts using *p*-anisaldehyde

Extract	Test Reagent	
	Anisaldehyde	Dragendorff
1	+ (Triterpene/flavonoids/glycosides)	- (No alkaloids)
2	+ (Flavonoids)	+ (Alkaloids)
3	+ (Triterpenes/glycosides)	+ (Alkaloids)

1: Leaf extract; 2: stem bark extract; 3: root extract

Conclusions

The present study shows interesting levels of activities of solvent extracts of different parts of *T. rhyncocarpum* against some of the bacteria tested (*M. vaccae*, *P. aeruginosa* and *B. subtilis*). The results provide some scientific rationale for the traditional use of the plant in Kenya to treat different microbial infections. Initial screening of the extracts suggests the presence of secondary metabolites derived from different biosynthetic pathways. Bioassay-guided fractionation, isolation and characterisation of the constituents are expected to identify the compounds and/or blends responsible for the observed activities.

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