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ANTIMICROBIAL ACTIVITY OF *CLEMATIS BRACHIATA* THUNB LEAF EXTRACTS

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ABSTRACT

The antimicrobial activity as well as phytochemical screening of the hexane, acetone, methanol and water extracts of *Clematis brachiata* Thunb (Ranunculaceae) leaves was investigated. The agar dilution assay method was used for the evaluation of the antimicrobial activity of extracts against 10 bacteria and four fungal species. The phytochemical screenings were performed by using the standard procedures. The acetone and methanol extracts were active against the 10 bacteria strains with MIC ranging between 1.0 and 3.0 mg/ml, whereas the water extract was only active against two Gram-negative bacterial strains at 10.0 mg/ml. There was no activity from the hexane extract. While there was complete growth inhibition by the acetone and methanol extracts against all the fungal species at 10 mg/ml, the hexane extract was active against all the fungal species except *Candida albicans* at 10 mg/ml. In contrast, the water extract did not show any activity against the fungal species. Phytochemical screening revealed the presence of phenols, tannin, saponin, flavonoids, terpenoids and glycosidic compounds and could be responsible for the above activities of the extracts. The results of this study support the traditional uses of this plant as antibiotics.

INTRODUCTION: Infectious diseases, caused by exposure to bacterial, fungal, viral and other microbial agents, constitute one of the main problems that modern medicine have faced over the last 30 years. Despite the high proportion of effective antibiotics available today, the emergence of resistant microorganisms has lowered their potency [1]. In addition, certain antibiotics have undesirable side effects while the emergence of previously uncommon infections is also a serious medical problem [2]. This has led scientists to search for new bioactive substances from various sources including medicinal plants.

Clematis brachiata Thunb (Ranunculaceae) locally known as Ityholo, is a deciduous climber that grows up to 5 m high. It is widely distributed in South Africa, Swaziland, Namibia and Botswana. It has a slender, twining woody stem and bears sasses of small, sweetly scented, creamy white flowers in the late summer and autumn. The decoction of the root is used in the treatment of malaria in Kenya and Tanzania [3, 4]. The infusion of the leaves and stem bark is used for treating schistomiasis in South Africa[5]. Ethnomedicinal information from the indigenous people of the Eastern Cape Province revealed that the leaf extract is also used as a remedy for eye infection, skin disorder and wounds [6].

Several species of the genus *Clamatis* have been widely used for folk medicine in many countries of the world. Examples include the decoction of the fruits and leaves of *C. vitalba* L. for the treatment of mouth

inflammation and rheumatic pain in Italy [7, 8] and the leaves of *C. drummondi* T & G used as a disinfectant and antibiotic in Mexico [9]. The antimicrobial activity of *C. hirsuta* Perr. & Guill. leaves and *C. vitalba* L [10, 11], antifungal activity of the aerial part of *C. drummondu* T & G. [12] as well as antibacterial activity of the aerial part of *C. cirrhosa* L. [13] have been reported. There is however, dearth of information on the antimicrobial activity of *Clamatis brachiata* leaves. Therefore, the aim of the present study was to evaluate the antibacterial, antifungal activities as well as phytochemical screening of the different solvent extracts of *C. brachiata* leaves.

MATERIALS AND METHODS:

Plant material: *Clematis brachiata* was collected in April, 2008 from a natural population within the premises of the University of Fort Hare, Alice, South Africa. The plant was identified by Prof DS Grierson of the Department of Botany, University of Fort Hare. A voucher specimen (M. Mostafa med. 2008/1) was prepared and deposited at the Giffen Herbarium of the University.

Test organisms: Ten bacterial and four fungal species used in this study were laboratory isolates obtained from the Department of Biochemistry and Microbiology, University of Fort Hare. The bacterial species consisted of five Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidus, Bacillus cereus, Micrococcus kristinae,* and *Streptococccus faecalis*) and five Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Klebsiella pneumoniae* and *Serratia marcescens*). The four fungal species used include *Aspergillus niger, Aspergillus flavus, Penicilium notatum* and *Candida albicans*.

Preparation of extract: The dried leaves of the plant were pulverized and portions of 50 g each were separately extracted in hexane, acetone, methanol and water for 24 h on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The extracts were filtered using a Buchner funnel and Whatman no. 1 filter paper. The hexane, acetone and methanol extracts were evaporated to dryness under reduced pressure at 40^{o}C using a vacuum rotary evaporator (Laborot 4000-efficient, Heldolph, Germany), while the water extract was freeze-dried with Savant Refrigerated Vapor Trap (RVT4104, USA). Each extract was re-constituted in their respective solvents to give 50 mg/ml stock solution. This was then diluted to the required concentrations of 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.0 and 10 mg/ml before being used for the antimicrobial assay.

Phytochemical screening: Phytochemical screening of the extracts were carried out for alkaloids, saponins, tannins, flavonoids, anthraquinones, steroids, phenols and glycosides using the standard procedures described by Harborne [14], Trease and Evans [15] and Sofowara [16].

Antibacterial assay: Each bacterial species was maintained on nutrient agar plates and recovered for testing by sub-culturing in nutrient broth (Biolab No.2) for 24 h. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth [17, 18]. The bacteria were streaked in a radial pattern on the agar plates[19]. The plates were later incubated at 37^{o}C and examined after 24 and 48 h. Each treatment was performed in triplicate, and complete suppression of growth at a specific concentration of an extract was required for it to be declared active [20, 21]. The assay was carried out at 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.0 and 10 mg/ml of the extract. Blank plates containing either only nutrient agar or 2% hexane, acetone or methanol served as controls. Hexane, acetone and methanol have been reported to be non-toxic to the organisms at 2% [22]. Chloramphenicol and streptomycin were used as standards in the experiment.

Antifungal assay: All the fungal cultures were maintained on potato dextrose agar (PDA) (Biolab No 2) and recovered for testing by subculturing on PDA for 4 days at 25^{o}C. PDA plates were prepared by autoclaving before the addition of the extracts. Each extract was vortexed with molten agar at 45^{o}C to final concentrations of 0.1, 0.5, 1.0, 5.0, and 10 mg/ml and poured into petri dishes. Plates containing only PDA or PDA with the respective solvent served as controls. The prepared plates were inoculated with plugs (5 mm in diameter) obtained from the actively growing portions of the mother fungal plates and incubated at 25^{o}C for 5 days. The diameter of the fungal growth was measured and expressed as percentage growth inhibition [23, 24, 25, 26]. Due to the nature of *Candida albicans,* the organism was streaked radially like the bacteria.

Significant differences between the means of treatments and controls were measured and calculated using the LSD statistical test.

RESULTS AND DISCUSSION: Phytochemical screening of *C. brachiata* Thunb (Ranunculaceae) in **Table 1** revealed that all the solvent extracts except hexane contained tannin, saponin, flavonoids, terpenoids and glycosides. The hexane extract only contained steroid.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF THE LEAF EXTRACTS OF *C. BRACHIATA*.

Parameters	Acetone	Methanol	Hexane	Water
Tanins	++	++	-	++
Saponins	+	++	-	++
Alkaloids	-	-	-	-
Flavonoids	++	+	-	+
Terpenoids	+	+	-	-
Steriods	-	-	+	-
Glycosides	+	++	-	++

+ presence ; - absence of constituent

The minimum inhibitory concentrations (MIC) of hexane, acetone, methanol and water extracts of the plant's leaves against the tested bacteria are shown in **Table 2**. The acetone extract inhibited the growth of all the bacteria at MIC range of 2.0 to 3.0 mg/ml. The methanol extract also suppressed the growth of all the organisms at inhibition range of 1.0 to 3.0 mg/ml, whereas, the water extract was active only against two Gram-negative bacteria, *P. aeruginosa* and *S. flexneri* at 10.0 mg/ml. Similarly the hexane extract showed no activity against the tested bacteria even at the highest concentration of 10.0 mg/ml.

The activity of the acetone and methanol extracts against all the tested Gram-positive and Gram-negative bacteria is noteworthy. Many secondary metabolites such as saponins, tannins, flavonoids and terpenoids from plant sources have been reported for their antimicrobial activity [27, 28, 29].

The phytochemical analyses have indicated the presence of the above phytochemical constituents in the methanol and acetone extracts could be responsible for their antimicrobial property. Generally, Gram-negative bacteria have been reported to have resistance to many of the antibiotics currently available in the market [30, 31].

Staphylcoccus aureus and *S. epidermidis* are the leading cause of superficial infections such as keratitis, conjunctivitis, dacryoadenitis and skin disorders [32, 33]. It is however, interesting to note that the acetone and methanol extracts of this plant showed appreciable activity against *S. aureus* and *S. epidermidis*. The broad spectrum activity of the extracts of *C. brachiata* leaves against all the tested bacteria might justify the use of this plant for the treatment of eye infection and skin disorders.

TABLE 2: ANTIBACTERIAL ACTIVITY OF THE ACETONE, METHANOL AND WATER EXTRACTS OF THE LEAVES OF *CLEMATIS BRACHIATA*

Bacteria	Minimum inhibitory concentration (mg/ml)					
	Gram +/-	Acetone	Methanol	Water	Chloramphenicol (µg/ml)	Streptomycin (µg/ml)
Staphylococcus aureus	+	2.0	2.0	na*	<2	<2
Staphylococcus epidermidis	+	2.0	2.0	na*	<2	<2
Bacillus cereus	+	2.0	2.0	na*	<2	<2
Micrococcus kristinae	+	2.0	2.0	na*	<0.5	<2
Streptococcus faecalis	+	2.0	2.0	na*	<2	<4
Echerichia coli	-	2.0	2.0	na*	<2	<2
Pseudomonas aeruginosa	-	3.0	3.0	10.0	<10	<2
Shigella flexneri	-	2.0	1.0	10.0	<2	<2
Klebsiella pneumoniae	-	2.0	2.0	na*	<2	<2
Serratia marcescens	-	2.0	2.0	na*	<2	<2

na* not active at 10 mg/ml.

The results of the antifungal assay of *C. brachiata* are presented in **Table 3**. The acetone and methanol extracts showed antimycotic activity against the organisms at concentration range of 0.01 to 1.0 mg/ml. Only the acetone extract showed complete inhibition against all the fungal species at 10 mg/ml. The methanol extract showed complete growth inhibition against all the fungal species except *A. flavus* (71.48%) at 10 mg/ml. But the hexane extract was also

moderately active against all the fungal species only at the concentration of 10 mg/ml, while the water extract did not show any activity against the fungi in this study.

TABLE 3: ANTIFUNGAL ACTIVITY OF THE ACETONE, METHANOL AND HEXANE EXTRACTS OF THE LEAVES OF *CLEMATIS BRACHIATA.* N = 3

Concentration (mg/ml)	Growth inhibition (%)			
	A. niger	*A. flavus*	*P. notatum*	*C. albicans*
Acetone extract				
10	100.00^{f}	100.00^{d}	100.00^{d}	100.00
5	61.39^{e}	43.89^{c}	70.00^{c}	0.00
1	59.17^{d}	28.61^{b}	54.44^{b}	0.00
0.5	54.72^{c}	0.00^{a}	0.00^{a}	0.00
0.1	51.67^{b}	0.00^{a}	0.00^{a}	0.00
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00
LC_{50}	0.10	5.54	0.92	-
Methanol extract				
10	100.00^{d}	71.48^{f}	100.00^{d}	100.00
5	60.83^{c}	63.89^{e}	56.94^{c}	0.00
1	59.17^{c}	58.33^{d}	54.17^{b}	0.00
0.5	54.72^{c}	53.33^{c}	0.00^{a}	0.00
0.1	51.67^{b}	44.72^{b}	0.00^{a}	0.00
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00
LC_{50}	0.37	0.35	0.92	-
Hexane extract				
10	63.30^{b}	59.26^{b}	56.67^{b}	100.00
5	0.00^{a}	0.00^{a}	0.00^{a}	0.00
1	0.00^{a}	0.00^{a}	0.00^{a}	0.00
0.5	0.00^{a}	0.00^{a}	0.00^{a}	0.00
0.1	0.00^{a}	0.00^{a}	0.00^{a}	0.00
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00

Values within a column followed by the same superscript are not significantly different at $p< 0.05$.

The acetone, methanol and hexane extracts were found to have broad spectrum activity against *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum* and *Candida albicans*. The susceptibility of *A. flavus* to the extracts of *C. brachiata* is also noteworthy, as this fungus is the most common cause of superficial infection and the second leading cause of invasive aspergillosis [34, 35].

Particularly common clinical syndromes associated with *A. flavus* are chronic granulomatous sinusitis, keratitis, cutaneous aspergillous and wound infections [36]. The fact that the organic extracts of *C. brachiata* leaves showed significant antifungal activity against all the fungal species may be explored in the management of some infectious mycosis.

Therefore, the antimicrobial activities of this plant extracts may justify its traditional uses for the treatment of eye infection, skin disorders and wounds.

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