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Ethnobotany and Antimicrobial Activity of *Gouania longispicata* Engl.

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ABSTRACT

Background: *Gouania longispicata* Engl. (Family: Rhamnaceae) has been used for traditional medicine applications for the treatment of over 42 ailments including allergy, tooth decay, mastitis and syphilis. The common use of *G. longispicata* in traditional healthcare systems echoes its relevance in light of modern pharmaceutical perspectives.
Aim: To determine the ethnobotany and antimicrobial activity of *G. longispicata*, which is traditionally used in Bwambara sub-county.
Materials and Methods: A cross-sectional study was conducted from January to June 2018, using questionnaires to collect ethnobotanical data on *Gouania longispicata* Engl. Air dried powdered plant material was sequentially extracted using hexane, chloroform, methanol and water. Phytochemical screening and antimicrobial activity were done following standard procedures.
Results: Phytochemical screening revealed the presence of flavonoids, cardiac glycosides, saponins, steroids, resins, and phenolic compounds in different plant extracts. Methanol fraction inhibited growth of all bacterial strains under study, while aqueous extract and aqueous fraction showed activity against the fungal strains under study and only one bacterial strain *Streptococcus pneumoniae*. The most susceptible microorganism was *Streptococcus pneumoniae* with MICs of 1.95 mg/mL for methanol fraction, 15.6 mg/mL for aqueous fraction and 31.25 mg/mL for aqueous extract.
Conclusion: The local population has used *G. longispicata* to treat various ailments for a long time. The antimicrobial activities of the various extracts provide a baseline for its use in the treatment of various diseases. Toxicity and structure elucidation of the bioactive compounds in the various extracts need to be studied.

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Introduction

Since ancient times, medicinal plants have been used in many cultures of the world as a source of medicine to treat diseases [1]. Despite the extensive use of antibiotics and vaccination programs, antimicrobial resistance has been a growing threat to the effective treatment of an ever-increasing range of infections caused by bacteria and fungi [2]. Antimicrobial resistance has been complicated by the emergence of HIV/AIDs, which renders the victims immune-compromised and open to opportunistic infections such as candidiasis, tuberculosis and typhoid, among others [3]. This calls for new sources of antimicrobial agents, such as indigenous medicinal plants. Much as the advent of conventional medicine over the past century has challenged the use of herbal medicine due to lack of scientific evidence [4], the common side effects of chemical drugs, lack of curative modern therapies for numerous chronic diseases and microbial resistance have led to resurgence of the use of medicinal plants [5]. Previous studies in Uganda have reported various plant species used in the treatment infectious diseases [6-9], including *G. longispicata* [8-9].

G. longispicata (family: Rhamnaceae) also known as "*omufururа*" in Runyankole/Rukiga, has the greatest diversity in Africa, Madagascar and Indian Ocean islands, Southern Asia, the Americas and Hawaii. In Africa, it is widely distributed in Nigeria, Democratic Republic of Congo (DRC), Sudan and East Africa. In Ethiopia, its leaves are harvested locally from the wild for the treatment of oral thrush [10]. In Rwanda, *G. longispicata* leaves are crushed, mixed with water and the juice extracted is administered orally to treat foetal trouble [11]. The Mbuti pygmies make young children drink it so that they grow strong [12]. In the DRC, it is used in the treatment of babesiosis and constipation in ethnoveterinary [13]. *G. longispicata* is also a preferred food by mountain Gorillas [14-16]. In Uganda, the leaves of *G. longispicata* are used to treat stomach ache [8]. Around Bwindi Impenetrable National Park, both leaves and roots are used by traditional birth attendants and herbalists to treat various conditions [17]. In Bwambara sub-county, *G. longispicata* was reported to treat over 42 ailments including allergy, urinary retention, syphilis, tooth decay, sore throat, wounds, itchy eyes, inflammations, skin infections, mastitis, worms, headache, asthma, body weakness, itchy body, colic pains, among others [9]. All these reported uses depict the high medicinal potential of *G. longispicata*, which may be as a result of various secondary metabolites in it. With increasing cases of antimicrobial resistance against allopathic medicines [2], the continuous use of *G. longispicata* by an individual for a long time may or may not affect its effectiveness, since the active ingredients in medicinal plants are secondary metabolites which have chemical structures like allopathic medicines. Since most resistance is usually to specific drug molecules, resistance to plant extracts is not as common since the various secondary metabolites in plants work in synergy.

This study investigated the antibacterial and antifungal activities of leaf extracts of *G. longispicata* against the microorganisms; *Staphylococcus aureus* [American Type Culture Collection (ATCC) 25923], *Streptococcus pneumoniae* (ATCC 51916), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 90028) and *Aspergilus flavus* (ATCC 22546). The test bacteria and fungi were selected on the basis of the reported diseases against which *G. longispicata* is used in ethnomedicine [9-10]. Classes of compounds found in the different extracts were also determined. The study also surveyed the duration and change in usage of *G. longispicata* in a bid to predict its effectiveness.

Materials and methods

Ethnobotany of *Gouania longispicata* Engl.

The ethnobotanical study was conducted in Bwambara sub-county from January to June 2018. The choice of the study area was linked to the ecological distribution of *G. longispicata*. A cross-sectional study was conducted to collect data from 134 participants using questionnaires and open interviews. Purposive sampling was employed on the basis that the participant should be using or should have ever used *G. longispicata*. Ethical approval was granted by Mbarara University of Science and Technology Research Ethics Committee (MUST-REC), number 19/08-17 and Uganda National Council for Science and Technology (UNCST), number NS34ES.

Plant collection and identification

The fresh plant materials (leaves) of *G. longispicata* were collected from Rukungiri district, Bwambara sub-county at latitude: -0.584175, longitude: 29.795473. Plant identification was done by a taxonomist in the department of Biology, Mbarara University of Science and Technology, where voucher specimen (GH18-002) were deposited.

Preparation of Plant Extracts

The plant leaves were air dried and ground into a fine powder using an electric grinder. In ethnomedicine, *G. longispicata* leaf powder is drunk in hot porridge or water as tea [9]. To mimic this usage, hot maceration of the powdered material was used to obtain the aqueous extract. This was done by soaking 200 gm of

powdered material in 1L of hot distilled water and allowing to mix for 12 hours using an electric shaker. The filtrate was obtained using a muslin cloth followed by Whatman filter paper No. 1 in a Buchner funnel with suction pump.

The hexane, chloroform and methanol fractions were obtained by soxhlet extraction. The powdered plant material (200 gm) was sequentially extracted with hexane, chloroform and methanol, according to increasing polarity of the solvents using soxhlet apparatus for 24 hours at a temperature not exceeding the boiling point of the respective solvent. The aqueous fraction was obtained from the residue from soxhlet extraction by maceration, because water is less volatile and could not be used for soxhlet extraction. The residue was dried and then hot maceration used to obtain aqueous fraction, using the same procedure as for aqueous extract. The filtrates and soxhlet extracts were concentrated by evaporation under reduced pressure using a rotary evaporator. A freeze drier was used to obtain dry extracts. All dried crude extracts were stored in sterile plastic bottles at 4˚C until use [18].

Phytochemical screening

Phytochemical screening of the crude extracts was done following standard methods to determine the presence of different classes of compounds; alkaloids, flavonoids, tannins, cardiac glycosides, steroids, saponins, resins and quinines [19-20].

Antimicrobial assay of *G. longispicata* extracts

The plant extracts were reconstituted with 10 % sterile dimethyl sulfoxide (DMSO) to make a solution of 500 mg/mL. The antimicrobial activities of the extracts were assessed using agar well diffusion method and broth tube dilution method [21].

Media preparation and sterilization

Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) were used for developing surface colony growth for bacterial and fungal assays respectively. MHA, PDA and Brain Heart Infusion (BHI) Broth were prepared according to manufacturers' instructions. The prepared MHA and PDA media were then dispensed into sterile glass petri dishes, allowed to cool and solidify under sterile conditions. The plates were then incubated for 24 hours at 37˚C to test for sterility.

Test organisms and agar well diffusion

The test organisms were standard strains [American Type Culture Collection, (ATCC)]; *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae* (ATCC 51916), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 90028) and *Aspergilus flavus* (ATCC 22546) obtained from Microbiology laboratory of Mbarara Regional Referral Hospital. The cultures of test organisms were prepared to a density of 0.5 McFarland standard.

Bacterial cultures were inoculated evenly onto solidified Muller Hinton agar and for by sterile cotton swab, while for fungi, inoculation was done on solidified PDA. For *Streptococcus pneumoniae*, inoculation was done on Muller Hinton agar supplemented with 5% sheep blood. On each sterilized media plate, equidistant wells (6 mm diameter and 2 mm apart) were made using sterile cork borer. An aliquot of 50 µl of each extract (500 mg/mL) was aseptically introduced into a respective agar well using micropipettes. Ciprofloxacin (0.02 mg/mL) and amphotericin B (5 mg/mL) were used as positive controls for bacteria and fungi respectively. DMSO (10 %) was used as negative control. The plates were incubated at 37˚C for 24 hours for bacteria and at 28˚C for 48 hours for fungi following standard methods [22]. The diameter of the inhibition zone (mm) was measured using a vernier. The experiments were performed in triplicate.

Determination of minimum inhibitory concentration (MIC)

The test was performed using agar well diffusion and broth tube dilution methods [23]. In agar well diffusion, each extract solution (500 mg/mL) was two-fold serially diluted to give concentrations of: 250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.63 mg/mL, 7.81 mg/mL, 3.95 mg/mL and 1.95 mg/mL. 50 µL of each extract and each concentration was aseptically introduced into a respective agar well. The inhibition zone was measured after 24 hours incubation at 37˚C for bacteria and after 48 hours incubation at 28˚C for fungi. The minimum concentration that inhibited growth was considered as MIC value of the extract.

In broth tube dilution, the 500 mg/mL extract was serially diluted as described above and 20 µL of a standard suspension of the test organism was then added to each concentration of the extract. The tubes were incubated at 37˚C for 24 hours for bacteria and at 28˚C for 48 hours for fungi. Then 50 µL of 0.2 mg/mL p-iodonitrotetrazolium chloride (Sigma-Aldrich, USA) was added in all tubes and incubated for 30 minutes at 37˚C. Viable microbial growth reduced the yellow dye (INT) to pink, while no color change indicated no growth. The lowest concentration, at which there was no color change and exhibited complete inhibition of microbial growth, was also regarded as MIC value of the extract [24].

In both agar well diffusion and broth tube dilution methods, two controls were made. A **medium control** contained media and test organism but without extract was done to find out whether the

media supported growth of organisms and whether the organism was viable. A **sterility control** contained media and extract but without the inoculum, and aimed at testing for sterility of the media.

Determination of the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

MBC and MFC were determined by sub-culturing the test dilutions used in MIC, on to fresh sterile solid media and incubate further for 24 hours for bacteria and 48 hours for fungi. The highest dilution or least concentration that yielded no single bacterial or fungal colony was taken as MBC or MFC respectively.

Data analysis

Ethnobotanical data was analyzed using Statistical Package for Social Scientists (SPSS) version 20 software package. Categorical variables were summarized as frequencies and percentages. Measurements of inhibition zones and respective concentrations were entered into SPSS-20 software for analysis.

Results and discussion

Ethnobotanical survey on *G. longispicata*.

Socio-demographic characteristics of the respondents

There were more female respondents (53.0 %) (Table 1). The participants were grouped according to age as youth (< 30 years), middle age (30 – 50 years) and old age (> 50 years) [25-26]. There were more users of *G. longispicata* who belonged to the old age category (47.8 %). The participants had little formal education, majority of whom had attended primary level only (61.2 %), while only 12.7 % had attended higher levels than primary level, including secondary and tertiary. This is in agreement with other previous ethnobotanical studies done in Uganda [9] and in Zimbabwe [27]. Only three of the interviewed participants were formally employed; two as primary school teachers and one as a private nurse. This reflects high poverty levels, leading to inability to buy allopathic medicines hence relying on medicinal plants for meeting the increasing health care needs. Low levels of education can also limit knowledge on conservation of flora which in turn hinders cultivation of medicinal plants.

Table 1. Demographic characteristics of participants

Characteristic		Number of informants (n = 134)	Percentage (%)
Sex	Female	71	53.0
	Male	63	47.0
Marital status	Single	5	3.7
	Married	105	78.4
	Divorced	3	2.2
	Widowed	21	15.7
Age group	<30	14	10.4
	30-50	56	41.8
	>50	64	47.8
Education	None	35	26.1
	Primary level	82	61.2
	Secondary level	13	9.7
	Tertiary	4	3.0
Occupation	Peasant	120	89.6
	Businessman	7	5.2
	Teacher	2	1.5
	Nurse	1	0.7
	Builder	1	0.7

Usage of G. longispicata as a medicinal plant.

More participants (46.3 %) reported to have used *G. longispicata* for over 10 years (Table 2). Majority of

the participants (53.0 %) reported that *G. longispicata* is still efficient, of which 32/71 (45.1 %) have used it for over ten years. Also, 33.6 % of the participants reported to have recognized an increase in its effectiveness with time, of which more than a half (24/45) have used it for over 10 years. This shows that *G. longispicata* is a trusted herbal remedy, which depicts high healing potential. This is also reflected a previous study which reported the use of *G. longispicata* to treat over 42 ailments in Uganda [9] and other ailments in Ethiopia [10]. Conversely, only two participants (1.5 %) who have used *G. longispicata* for less five years reported that it had lost its effectiveness. Unlike allopathic medicines, the continuous use *G. longispicata* may not affect its effectiveness over some time, since like other medicinal plants it contains a variety of active secondary metabolites. Majority of the participants, 65.7 % reported forest reserve as the main source for collection of *G. longispicata* while only one participant had it cultivated.

Table 2. Evaluation of use of *G. longispicata* by local population

Characteristic	Description	Duration of use (years)	Frequency (n =134)	Percentage
Used *G. longispicata* for how long?		<5	52	38.8
		5-10	16	11.9
		>10	62	46.3
Any change in effectiveness on continuous use of *G. longispicata*?	Works as before	<5	29	
		5-10	10	
		>10	32	
		Subtotal	**71**	**53.0**
	There is an increase	<5	15	
		5-10	5	
		>10	24	
		Subtotal	**45**	**33.6**
	There is a decrease	<5	3	
		5-10	0	
		>10	2	
		Subtotal	**5**	**3.7**
	No longer effective	<5	2	
		5-10	0	
		>10	0	
		Subtotal	**2**	**1.5**
Source of *G. longispicata*	Forest reserve		88	65.7
	National Park		5	3.7
	Wild		9	6.7
	Cultivated		1	0.7
	Near rivers		12	9.0
	Valleys		2	1.5

Antimicrobial activity of *G. longispicata* leaf extracts

The inhibition zones ranged from 8.50±0.83 to 24.00±0.82 mm (Table 3). The methanolic fraction inhibited the growth of both gram-negative; *Staphylococcus aureus*, *Streptococcus pneumoniae* and gram-positive bacteria; *Escherichia coli, Pseudomonas aeruginosa,* but had no activity on fungi. Previous studies on a plant in the same genus, *Gouania longipetala*, reported ethanolic extract to inhibit the growth of both gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) [28], just like the methanolic fraction in the current study.

Table 3: Mean (mean ± standard deviation) microbial growth inhibition zones in agar well diffusion method treated with 500 mg/mL of plant extracts (mm).

Microorganism	Plant extracts					Drug
	n-Hexane fraction	*Chloroform fraction*	*Methanol fraction*	*Aqueous fraction*	*Aqueous Extract*	

Gram-positive bacteria:						
Staphylococcus aureus	-	-	12.78±0.42	-	-	23.50±0.41
Streptococcus pneumoniae	-	-	20.00±0.00	17.50±0.41	24.00±0.82	19.50±0.50
Gram-negative bacteria:						
Escherichia coli	-	-	12.44±0.62	-	-	15.83±0.62
Pseudomonas aeruginosa	-	-	8.50±0.83	-	-	22.23±0.12
Fungi:						
Candida albicans	-	-	-	16.67±2.69	13.75±0.20	24.00±1.00
Aspergilus flavus	-	-	-	13.33±2.78	9.00±0.82	24.00±1.00

The aqueous fraction and aqueous extract inhibited growth of *Streptococcus pneumoniae* and the two fungi strains; *Candida albicans* and *Aspergilus flavus*. The aqueous extract gave a larger inhibition zone (24.00±0.82 mm) than the aqueous fraction (17.50±0.41 mm) against *Streptococcus pneumoniae* probably because some of the active components against the same organism were removed by methanol for the aqueous fraction, since the aqueous fraction was obtained from the methanol residue. However for fungi, the aqueous fraction showed larger zones of inhibition than the aqueous extract, probably because the sequential treatment of the plant material with n-hexane, chloroform and methanol could have broken the cell walls to facilitate extraction with water. The n-hexane and chloroform fractions did not inhibit growth of any of the test organisms. The largest diameter of zone of inhibition, 24.00±0.82 mm was given by the aqueous extract against *Streptococcus pneumoniae*. The drugs, ciprofloxacin (0.02 mg/mL) and amphotericin B (5 mg/mL) used as positive controls for bacteria and fungi respectively, showed sensitivity for all organisms.

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The determined MIC values for different extracts on different microorganisms ranged from 1.95 to 250 mg/mL (Table 4). MIC determination is vital in diagnostic laboratories because it aids in confirming resistance of microorganisms to an antimicrobial agent and also helps to monitor the activity of new antimicrobial agents [22]. The highest antibacterial activity was exhibited by the methanolic fraction, with the lowest MIC of 1.95 mg/mL against *Streptococcus pneumoniae*. This high activity supports the reported ethnomedical uses of *G. longispicata* in treatment of various bacterial infections caused by this strain such as respiratory disorders [9]. The aqueous fraction showed better activity on *Candida albicans* with MIC of 31.25 mg/mL and MFC 62.5 mg/mL, which supports the reported use of *G. longispicata* water extracts in the treatment of fungal infections such as oral thrush [10] and mastitis [9].

Table 4. MIC, MBC and MFC (mg/mL) performance of different extracts of *G. longispicata* against pathogenic organisms

Microorganism		**Methanol fraction**	**Aqueous fraction**	**Aqueous Extract**
Bacteria:				
Staphylococcus aureus	MIC	7.81	-	-
	MBC	62.5	-	-
Streptococcus pneumoniae	MIC	1.95	15.625	31.25
	MBC	3.91	31.25	62.5
Escherichia coli	MIC	125	-	-
	MBC	250	-	-
Pseudomonas aeruginosa	MIC	125	-	-
	MBC	250	-	-
Fungi:				

Candida albicans	MIC	-	31.25	125
	MFC	-	62.5	250
Aspergilus flavus	MIC	-	125	250
	MFC	-	250	500

The observed activity of *G. longispicata* against: *Staphylococcus aureus* supports the reported use to treat skin infections, respiratory disorders and wound infections [9]; *Escherichia coli* supports use to treat anorexia and stomach disorders [8-9]; *Pseudomonas aeruginosa* supports use to treat inflammations, respiratory disorders, limb and joint infections and gastrointestinal infections [9-10]; and *Aspergilus flavus* supports use to treat respiratory illnesses, ear and eye infections [9]. The findings demonstrated that a single extract could be active on different organisms with different MIC values, leading to conclude that the active secondary metabolites exerting the microbial inhibition are different in that particular extract, which is in agreement with the previous studies [29]. In the present study, the MIC values were lower than MBC and MFC values. This suggests that the plant extracts were bacteriostatic at lower concentrations but bactericidal and fungicidal at higher concentrations [22].

Phytochemical composition of *G. longispicata*

The different extracts were qualitatively analyzed for phytochemicals. n-hexane fraction showed the presence of steroids, methanol fraction contained cardiac glycosides, steroids, flavonoids, saponins and phenolics. The aqueous fraction contained flavonoids, resins, saponins and phenolics. The aqueous fraction contained flavonoids, resins, saponins and phenolics (Table 5). Phytochemical screening of some plants in the same genus Gouania like *Gouania longipetala* showed the presence of phenolics, reducing sugars, phytosterols, triterpenoids,saponins and flavonoids, which are related to the findings of current study [28].

Table 5. Phytochemical composition of the leaf extracts of *G. longispicata*

Plant secondary metabolite	*G. longispicata* leaf extract				
	n-Hexane fraction	Chloroform fraction	Methanol fraction	Aqueous fraction	Aqueous Extract
Alkaloids	-	-	-	-	-
Cardiac glycosides	-	-	+	-	-
Flavonoids	-	-	+	+	+
Resins	-	-	-	+	+
Saponins	-	-	+	+	+
Steroids	+	-	+	-	-
Phenolics	-	-	+	+	+
Quinines	-	-	-	-	-

(+), test gave positive results; (-), test gave negative results.

The presence of these phytochemicals are linked to the observed antimicrobial activity and as well as the traditional use of *G. longispicata*. For instance, previous studies on flavonoids have reported anti-inflammatory properties [30-31], antiallergenic [30], wound healing [32-33]. Studies of phenolic compounds have reported anti-inflammatory properties [34-35]. Saponins have been reported to exhibit antifungal properties [36], wound healing potential [37-39].

Conclusion

The findings revealed much faith in the effectiveness of *G. longispicata* in treatment of various ailments. This claim was also supported by the presence of different phytochemicals within different plant extracts which included; flavonoids, cardiac glycosides, saponins, steroids, resins, and phenolic compounds.

The results of the current study suggest that the extracts of *G. longispicata* can be used as potential leads to discover new broad spectrum antimicrobial drugs, since they were active on both bacteria and fungi. Therefore, further chemical analysis of the

aforementioned plant extracts should be done to elucidate the structures of the bioactive compounds responsible for the observed antimicrobial activity. Furthermore, the bioactive compounds need to be subjected to pharmacological analysis with the aim of assessing their in vivo efficacy, toxicity, interactions and contraindications.

The findings of the current study support the traditional use of *G. longispicata* in the treatment of various infectious diseases. However, since the bioactivity of an alcohol (methanol) extract differs from the aqueous extract, the use of local brew (*Tonto* in *Runyankole/Rukiga*), which contains both alcohol and water would be a better extracting solvent for local use.

Conflict of interest

The authors declare that there are no competing interests.

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