Comparative Phytochemical and Antimicrobial Evaluation of the Shoot and Root of *Mariscus ligularis* (L.) Urb. (Cyperaceae) Crude Extracts

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Abstract
Mariscus ligularis (L.) Urb. has been traditionally reported for healing and antimicrobial properties. In order to evaluate and scientifically prove the medicinal values of the parts used, qualitative and quantitative phytochemical and antimicrobial studies were carried out on the shoot and root of this sedge. Aqueous and ethanolic crude extracts of the shoot and root of *M. ligularis* were tested against fungi: *Aspergillus niger* and *Candida albicans* and bacteria: *Staphylococcus aureus* and *Salmonella typhi.* Antimicrobial activity evaluation was carried out at 50 mg/mL and 100 mg/mL concentrations by the Agar Well Diffusion method. Standard antibiotics – Clotrimazole (antifungal) and Ciprofloxacin (antibacterial) were used as controls.
Phytochemical screening indicated the presence of phenols, flavonoids, reducing sugars, tannins and saponins in varying quantities; steroids and alkaloids were absent. The crude ethanolic extracts were more active than the aqueous extracts against the bacteria than the fungi. The shoot ethanolic extract was the most effective against *S. aureus* (37.13 ± 0.38 at 100 mg/mL and 33.63 ± 0.88 at 50 mg/mL). The crude shoot extracts were generally more effective than the root extracts. From these findings, crude ethanolic extracts of the shoot of *M. ligularis* exhibited a good potential source of new drugs upon isolation and purification of its bioactive compounds for treating infections caused by these pathogens and particularly drug evasive *C. albicans*.

Keywords: antimicrobial, crude extracts, *Mariscus ligularis*, phytochemical

Introduction

The significance of the use of plants in primary healthcare in both rural and urban Nigeria has been stressed (Okigbo *et al.,* 2009). Traditional medicine is not only popular in Africa but across the world because of its significance in primary healthcare (Elujoba *et al.,* 2005). More than 80% of Nigerians have been reported to use herbal medicine to cure ailments since less than 35% of the population has access to modern healthcare facilities (Okigbo *et al.,* 2009).

This high percentage usage in Nigeria is due to their relative safety compared to synthetic drugs (Iwu *et al.,* 1999), availability and the efforts in the Nigerian media; with the print media dedicating sizeable sections of their papers to traditional medicine. Newspapers such as "The Guardian" and "Punch" have traditional medicine sections that enlighten the public on the usefulness and efficacy of the easy-to-access herbal remedies. The electronic media and online advertisement as well as e-tradomedicine have also contributed immensely to the recent interests of Nigerians in herbal medicine.

One of the most readily available plants, often neglected being a weed, is *Mariscus ligularis* L. (Urb.) of the family Cyperaceae. *M. ligularis* is a common plant found growing wild in Nigeria particularly in mangrove swamps, brackish water and lagoons except in the far north of the country. This species is a stout tufted plant, one metre or more tall, with thick fibrous roots. The leaf sheath is red at the base, 1 m long and 1 cm wide at the base, tapering gradually to a sharp tip and stiff with cutting edges. Inflorescence is a compound umbel in structure subtended by blue-green leafy bracts nearly as long as the leaves. Spikelets are normally 5 mm long and 2 mm broad, which are composed of 3 or 4 florets. The stem is not perfectly triangular in shape but it is greenish in colour (Lowe and Stanfield, 1974; Burkill, 1985).

Ethnobotanically, the stem is reported to have a healing effect when chewed and bandaged on a cut or wound for at least 3 days. The ground swollen stem, when mixed with honey, is used in treating gonorrhea while the rhizome is used as a condiment (Burkill, 1985). Much of the phyto-medicinal values of *M. ligularis* have not been scientifically investigated nor reported in spite of its abundance in the coastal lagoonal areas of Nigeria and the widely reported irritation of its inflorescence (Burkill, 1985). The concentrations of the bioactive compounds in different parts of the plant have not been investigated

and this is needed in order to guide users in targeting the parts with the highest concentration for therapeutic and pharmacognostic uses. Therefore, the primary aim of this research is to evaluate and compare the medicinal values of the shoot and root of *M. ligularis* so as to locate where the bioactivity is more concentrated.

Materials and Methods

Sources of Plant Material and Test Microbes

M. ligularis plant was collected within the University of Lagos, Akoka, Lagos State, Nigeria. The taxonomic identification and authentication were done at the University of Lagos Herbarium (LUH 5884). The test fungi, *Aspergillus niger* and *Candida albicans* and the test bacteria, *Staphylococcus aureus* and *Salmonella typhi* were sourced from the Department of Microbiology, University of Lagos. The fungi were maintained on Saboraud Dextrose Agar (SDA) at 4 ºC while the bacteria strains were maintained on Mueller Hinton agar (MH) at 4 ºC.

Preparation of Plant Extracts

The plant materials (root and shoot) were cut into pieces with the aid of a knife and oven-dried at 40 ºC for a period of 1 week. Dried samples were ground into powder using a grinding machine. For the ethanolic and aqueous extractions, 100 g of the oven-dried powdered plant material was weighed and soaked in 500 mL of ethanol and distilled water, respectively, in 4 sterilised conical flasks and left undisturbed for 48 hours. The extracts were then filtered off using Whatmann No. 1 filter paper. The filtrates were concentrated under vacuum using a rotary evaporator below 40 ºC (Bag *et al.,* 2009).

The preparation of 100% (100 mg/mL) extract concentrates was done by mixing 100 mg of the concentrated extract with 1 mL of the respective solvents (ethanol and distilled water). 50% (50 mg/mL) extract concentrates were prepared by mixing 50 mg of the concentrated extract with 1 mL of the respective solvents.

Phytochemical Analysis

The phytochemical constituents of the root and shoot of *M. ligularis* were evaluated qualitatively and quantitatively using the methods described by Harborne (1998). Screening involved tests for reducing sugars, alkaloids, cardiac glycosides, saponins, tannins, flavonoids, steroids and terpenoids.

Preparation of Culture Media

Sabouraud's Dextrose Agar (SDA) and Mueller Hinton agar (MH) were used for the isolation of the fungi and bacteria strains, respectively. To prepare these media, 31.5 g of SDA and 22.5 g of MH were each dissolved separately in 500 mL of distilled water in sterilised conical flasks. The mouths of the flasks were plugged with cotton wool, wrapped with aluminum foils and taped round in order to avoid contaminants. The flasks were shaken gently to prevent the formation of air bubbles and then the media were sterilised in an autoclave at 121 ºC for 15 minutes and allowed to cool before pouring them on the plates (Adeniyi *et al.,* 2014).

Antibacterial Activity Test

This was performed using the Agar Well Diffusion Test Method of Radhika and Aneja (2009). Base plates were prepared by pouring 10 mL Mueller Hinton (MH) agar into sterile petri dishes (9 cm) and allowing to set. The molten MH agar held at 48 ºC was inoculated with a broth culture (10^3 and 10^4 bacteria per mL) of test organism and poured over the base plates to form homogenous top layers. A cup borer of 9 mm was used to bore holes and about 0.2 mL of plant extracts was pippeted into the well. This was repeated for the plant's parts' extracts and the controls.

Two controls: Ciprofloxacin and sterile distilled water were used. The Ciprofloxacin control was prepared by dissolving 1 g of Ciprofloxacin powder in 10 mL of sterile distilled water. The plates were kept at room temperature for about 15 minutes and incubated at 37 ºC for 24 hours. Then the ratio of the inhibition zone (mm) produced by the extracts and the inhibition zone around the Ciprofloxacin reference (mm) was used to express antibacterial activity.

Antifungal Activity Test

The Agar Well Diffusion Method of Radhika and Aneja (2009) was also adopted. About 10 mL of Saboraud Dextrose Agar (SDA) was poured into petri dishes and allowed to solidify. Liquid inoculum was prepared by pouring cooled sterile distilled water on already grown fungal plates. The suspension was then transferred into a test tube. This was done in order to make the spores easily removeable from the pure culture. Serial dilution from 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} was then carried out. A syringe was used to transfer 0.5 mL of the aliquots into each of the plates. A sterile rod was used to gently stir the spores for even distribution in the plates. A cup borer of 9 mm was used to bore holes and about 0.2 mL of extracts was pippeted into the well. This was repeated for all the extracts and the controls.

Two controls: Clotrimazole and sterile distilled water were used. The Clotrimazole control was prepared by

dissolving 1 g of mycotine cream in 10 mL of the diluent. Afterwards, concentrations of 150 µg/mL and 75 µg/mL were prepared. All the plates containing the extracts and fungi were incubated at 25 ^{0}C then the zones of inhibition were measured after 48 hours of incubation.

Results

Qualitative Phytochemical Analysis

Phytochemical analysis carried out on the root and shoot of *M. ligularis* revealed the presence of tannins, flavonoids and cardiac glycosides. Alkaloids and steroids were absent in both extracts and all plant parts tested. The aqueous extracts of the root and shoot also showed the presence of saponins, which was also present in the shoot ethanolic extract but absent in the root ethanolic extract. The ethanolic extracts of the root and shoot showed the presence of terpenoids (Table 1).

Table 1: Qualitative Analysis of *M. ligularis* Extracts

Test	Inference			
	Root (aq)	Shoot (aq)	Root (eth)	Shoot (eth)
Reducing sugars	–	–	+	+
Alkaloids	–	–	–	–
Steroids	–	–	–	–
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	–	+	+
Cardiac glycosides	+	+	+	+
Saponins	+	+	–	+

Key: aq = aqueous extract; eth = ethanolic extract
– = Absent; + = Present

Quantitative Phytochemical Analysis

The ethanolic root extract possessed the highest percentage of reducing sugars (4.29%/100 g), which was absent in the aqueous extract. For tannins, the ethanolic root extract had the highest percentage (0.04%/100 g) followed by the aqueous shoot extract. The shoot and root extracts had saponins, however, water seemed to be a better solvent with aqueous root extract having higher saponin content (0.33%/100 g) than the ethanolic shoot extract. The ethanolic root extract lacked saponins. For the flavonoids, the ethanolic shoot extract (0.17%/100 g) and root extract (0.14%/100 g) had higher percentages of flavonoids compared to the aqueous extracts. Phenols were also present in the root and shoot extracts (Table 2).

Antibacterial Activity Test

The antibacterial activity tests carried out on the aqueous and ethanolic extracts of the root and shoot of *M. ligularis* showed that 100% and 50% concentrations of the ethanolic root and shoot extracts were strongly active against *Salmonella typhi* while aqueous extracts showed no activity (Table 3).

Table 2: Quantitative Phytochemical Analysis of *M. ligularis* Extracts

Phytochemical (%/100 g)	Extract			
	Root (aq)	Shoot (aq)	Root (eth)	Shoot (eth)
Tannins	0.018	0.028	0.049	0.013
Reducing sugars	**	**	4.293	3.098
Flavonoids	0.119	0.114	0.144	0.174
Phenols	0.018	0.018	0.013	0.021
Saponins	0.329	0.332	**	0.29

Key: aq = aqueous extract; eth = ethanolic extract
** = Not Detected

The inhibitory activities of the ethanolic root and shoot extracts increased at higher concentrations. Similarly, against *Staphylococcus aureus*, only the ethanolic root and shoot extracts showed inhibitory activities but not at both concentrations. The ethanolic root extract showed no activity at 50% concentration (Table 3). Of significant note is the higher inhibitory activity of the ethanolic shoot extract against *S. aureus* compared to the standard antibiotic Ciproflocaxin (Table 3).

Antifungal Activity Test

The antifungal activity tests carried out on the aqueous and ethanolic extracts of the root and shoot of *M. ligularis* showed that only 100% and 50% concentrations of the ethanolic shoot extract were active against *Aspergillus niger* while other extracts showed no activity at all concentrations (Table 4).

The inhibitory activity of the ethanolic shoot extract increased at higher concentrations. Similarly, only the ethanolic shoot extract showed strong activity against *Candida albicans* at both concentrations as compared to the control (Table 4). The inhibitory activity of the ethanolic shoot extract increased at higher concentrations.

Discussion

Okigbo *et al.* (2009) cited the description of alkaloids and their derivatives by Stray (1998) as being useful in medicine for their analgesic, anti-spasmodiac and bactericidal effects. *M. ligularis* lacked steroids and alkaloids since none of the tested extracts recorded them. However, the presence of flavonoids, phenols and glycosides in all the extracts was enough to annul the absence of steroids and alkaloids as plant antimicrobials. Dyer and Bower (2015) remarked that glycosides act against predation by microorganisms, insects and herbivores in plants. The tannins present

Table 3: Measurement of Antibacterial Inhibition Zones

Bacteria	Extracts	Zone of Inhibition (mm)	
		100%	**50%**
Salmonella typhi	Root (aq)	na	na
	Shoot (aq)	na	na
	Root (eth)	$15.25^{a} \pm 0.51^{b}$	$11.75^{a} \pm 0.25^{b}$
	Shoot (eth)	$21.25^{a} \pm 0.51^{b}$	$20.38^{a} \pm 0.38^{b}$
	Ciproflocaxin	$28.88^{a} \pm 0.13^{b}$	$26.88^{a} \pm 0.13^{b}$
	Water	na	na
Staphylococcus aureus	Root (aq)	na	na
	Shoot (aq)	na	na
	Root (eth)	$12.25^{a} \pm 0.51^{b}$	na
	Shoot (eth)	$37.13^{a} \pm 0.38^{b}$	$33.63^{a} \pm 0.88^{b}$
	Ciprofloxacin	$32.88^{a} \pm 0.13^{b}$	$28.63^{a} \pm 0.38^{b}$
	Water	na	na

Key: aq = aqueous extract; eth = ethanolic extract; na = No inhibition
[a] = mean of duplicates (including diameter of borer); [b] = standard error

Table 4: Measurement of Antifungal Inhibition Zones

Fungi	Extracts	Zone of Inhibition (mm)	
		100%	**50%**
Aspergillus niger	Root (aq)	na	na
	Shoot (aq)	na	na
	Root (eth)	na	na
	Shoot (eth)	$15.50^{a} \pm 0.51^{b}$	$13.88^{a} \pm 0.38^{b}$
	Clotrimazole	$28.88^{a} \pm 0.13^{b}$	$26.88^{a} \pm 0.13^{b}$
	Water	na	na
Candida albicans	Root (aq)	na	na
	Shoot (aq)	na	na
	Root (eth)	na	na
	Shoot (eth)	$15.38^{a} \pm 0.63^{b}$	$13.50^{a} \pm 0.25^{b}$
	Clotrimazole	$17.50^{a} \pm 0.25^{b}$	$15.88^{a} \pm 0.13^{b}$
	Water	na	na

Key: aq = aqueous extract; eth = ethanolic extract; na = No inhibition
[a] = mean of duplicates (including diameter of borer); [b] = standard error

in all the tested extracts may possess many physiological activities such as the stimulation of phagocytic cells, host-mediated tumour activity and a wide-range of anti-infective actions, as reported by Okigbo *et al.* (2009).

The presence of these six phytochemicals: reducing sugars, terpenoids, tannins, flavonoids, saponins and cardiac glycosides (Tables 1 and 2) are most likely to account for the high inhibitory activity of the ethanolic shoot extract. The reducing sugars in particular may be responsible for the antibacterial activity since they form a building block for the production of phytoalexins (Yukihiro *et al.*, 2002). Yukihiro *et al.* (2002) described phytoalexins as antimicrobial substances synthesised by plants, which accumulate rapidly at areas of incompatible pathogen infection. Further work needs to be done to determine the phytoalexins present in this plant through their isolation and purification. The presence of sugars in the ethanolic extract and absence in the aqueous extract may also explain the higher activity of the extracts against tested bacteria compared to the fungi.

The unproven report of Burkill (1985) that the stem of *M. ligularis* has a healing effect on wounds and that it is used to treat gonorrhoea maybe scientifically confirmed by the presence, in all extracts herein, of wound healing tannins and antimicrobial flavonoids, glycosides, phenols and reducing sugars.

The ethanolic shoot extract was the most active against *S. typhi* (100%: 21.25 ± 0.51 and 50%: 20.38 ± 0.38) and compared favourably with the control Ciprofloxacin (100%; 28.88 ± 0.13 and 50%: 26.88 ± 0.13). The isolation and purification of the bioactive compounds responsible for this inhibitory activity are necessary to produce drugs with less or no side effects, which can be used effectively against *S. typhi*. The ethanolic root extract also showed a mild inhibitory action against *S. typhi* (100%: 15.25 ± 0.51 and 50%: 11.75 ± 0.25). This further emphasises the

anti-microbial potency of the ethanolic extracts of this plant. The inactivity of the aqueous extracts against *S. typhi* demonstrates the suitability of ethanol as a good extraction agent (Table 3).

The high inhibitory activity of the ethanolic shoot extract against *S. aureus* (100%: 37.13 ± 0.38 and 50%: 33.63 ± 0.88), which was higher than the antibiotic control, Ciprofloxacin (150 µg/mL: 32.88 ± 0.13 and 75 µg/mL: 28.63 ± 0.38) (Table 3) is of particular significance. Though, the the root extract also had antibacterial activity in high concentration, at a lower concentration of 50%, it became inactive.

This shows that the concentration of the extract also determines their activity (Table 3). With the high potency of the ethanolic shoot extract against *S. aureus*, isolating the bioactive ingredients responsible for the inhibitory activity could lead to the production of a powerful and reliable antibiotic, more potent than Ciprofloxacin, specifically for controlling *S. aureus* infections in humans.

Aspergillus niger is one of the most common species of *Aspergillus* that cause aspergillosis in humans. Aspergillosis develops in the lungs of humans who are immuno-compromised and results in symptoms such as cough, fever, chest pains and breathing difficulties. In plants, it causes the black mould on onion bulbs. The minimal antifungal activity of the extracts against *A. niger* (Table 4) may be due to the mycotoxin called Ochratoxin produced by *A. niger* for self resistance against antifungal drugs (Samson *et al.,* 2004).

The resistance of *Candida albicans* to most antimicrobial agents has been variously reported and this is clearly demonstrated in this study with no aqueous extract of both plant parts showing any inhibitory activity against *C. albicans.* However, the ethanolic shoot extract showed significant inhibitory activity (100%: 15.38 ± 0.63 and 50%: 13.50 ± 0.25) against the "stubborn" fungus (Table 4). *C. albicans* is the causative agent of candidiasis; an opportunistic infection difficult to control in immuno-compromised patients. Of special note is the inhibitory activity of the ethanolic shoot extract against the evasive *C. albicans* whose infections have been reported as difficult to treat (Adeniyi *et al.,* 2014). Its values at both concentrations are very close to that of the control, Clotrimazole. This may indicate its use in combating the fungus.

Traditional medicine practitioners make use of water primarily as a solvent but studies have shown that alcohol extracts of plants are much better and powerful. This may be due to the enhanced solubility of the plants' active components in the organic solvent (De Boer *et al.,* 2005). The aqueous and ethanolic extracts used in this study showed either inhibitory or no activity against the different species of fungi and bacteria tested. The ethanolic extracts showed higher antimicrobial activities than the aqueous extracts. This implies that ethanol extracted more active phyto-constituents compared to water. This is in agreement with the reports of Nwachukwu and Uzeato (2010), Peni *et al.* (2010) and Adeniyi *et al.* (2014).

The fact that the aqueous extract did not inhibit as much as the ethanolic extract does not mean that the plant parts tested did not contain the sought-after phytochemicals because the extraction solvent is a strong determinant of its recovery even when present. The recorded higher inhibitory activities of the ethanolic shoot extract against all the test pathogens in this work indicate that the shoot contains more phytochemicals than the root. This information is important in guiding users of this plant in herbal medicine and pharmacognosy to recognise the part to target for the maximum and effective recovery of bioactive compounds.

Conclusion

The plant *M. ligularis* could be an important source of natural antimicrobials particularly against *S. aureus, S. typhi, A. niger* and *C. albicans.* The outstanding inhibitory activities against *S. aureus,* which is even higher than the standard antibiotic and the good action against the evasive *C. albicans* indicate that the plant contains compounds that can be used as antimicrobial agents in new drugs for the treatment of infectious diseases caused by these pathogens. *Mariscus ligularis* possesses compounds with more antibacterial than antifungal properties.

The most active extract (ethanolic shoot extract) is hereby recommended for isolation of the therapeutic antimicrobials and further pharmacological evaluation.

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