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Phytotoxic and antimicrobial activities of saponin extracts from lemon grass

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ABSTRACT: Phytotoxic activity of the Lemon grass saponins was tested on the germination of maize and bean seeds and on the growth of the germinated seedlings. The saponin extract inhibited the germination of maize and bean seeds and the growth of the shoot and root of the seedlings. The bean seeds were more sensitive to the saponin effect than the maize seeds because their growth was more inhibited. The inhibition was concentration-dependent in that the higher the saponin concentration the higher the inhibition of shoot or root-length.

Saponin extract from Lemon grass (Cymbopogon citratus) was tested on six pathogenic microbes namely Staphylococcus aureus Bacillus substilis, Escherichia coli, Pseudomonas aeruginosa, Candida albican and Aspergillus niger. The saponin extract inhibited the growth of all the microbes and was comparable to the effects produced by Ampicillin, a standard antibiotic. At 10% dose concentration, the saponin extract was 81% as effective as Ampicillin (30 mg/ml) inhibiting the growth of Escherichia coil

Gram- negative bacteria are more sensitive to the saponin extract because their growth was more inhibited. This report justifies the application of Lemon grass in traditional healthcare delivery in the West African sub region.

Key Words: Saponins; Lemon grass; Maize; Beans; Phytotoxic; Antifungal; Antimicrobial.

Introduction

Medicinal plants contain naturally occurring metabolites such as saponins, pelyphenols, haemagglutinins, oxalates, cyanides, nitrites and nitrates e.t.c (Mahato *et al*, 1992, Waterman, 1986).

Lemon grass is one of the medicinal plants commonly used in the West African subregion for treating or managing febrile illnesses in various communities.

Saponins are regarded as a group of allelochemical agents inhibiting plant and weed growth, as well as seed-germination (Oleszek *et al* 1993). The phytotoxic activity of saponins on seed germination and on plant growth results from their inhibitory effect on plant growth hormones like the Auxins and gibberellins (Waller, 1989, 1993). Phytoxicity is a phenomenon which can be applied in agriculture and could effectively be used in weed control, plant selection and herbicidal application in field experiments.

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The aim is therefore to report the effects of saponins extract from Lemon grass on the germination and the growth of bean and maize seeds and to examine the possible antibiotic effects of the saponins for other pharmacological activities in an attempt to justify its use in traditional medical folklore.

Materials and Methods

Fresh leaves of lemon grass were collected and identified by a Taxonomist in the Botany Department, University of Ibadan and the saponins was extracted with boiling petroleum ether (60^0 - 80^0C) and aqueous methanol in Soxhlet apparatus according to the method of Fenwick *et al* (1992). The extract was purified by separating funnel method to remove impurities like sugars, oligosaccharides, flavonoids. A portion of the crude methanolic extract containing saponin was weighed and taken up in methanol. This extract was partitioned between a 1:1 mixture of n-but anol and distilled water, using 60 ml of n-butanol and 60ml of distilled water in a separating funnel. The saponins passed into the lower layer of n-butanol which was then evaporated to dryness. The residue was taken up in methanol and this was the n-butanol extract used for further studies.

Healthy bean and maize seeds used for this study were bought from Bodija market, Ibadan.

Phytotoxic Activity

Preparation of Test Solutions

One gram of the extract was dissolved in 100ml of distilled water to get a 1% test solution from which 0.5%, 0.25% and 0.1% test solutions were prepared through serial dilutions.

Preparation of Maize and Bean Culture

Maize and bean seeds were cultured in petridishes with cottonwool placed at the bottom. Five petridishes were used for each of the maize and beans culture, 10ml of each of the test solutions was pippeted in to separate dishes so that the cotton wool was completely soaked. Four healthy seeds were placed in a circular pattern in each petridish and 10ml of distilled water was used as the control culture. About 10ml of distilled water was added to each culture everyday from the second day of culturing. The experiment was done in triplicates. The shoots and the root length were measured in centimeter after six days of culturing.

Antimicrobial Activity

Preparation of Culture Media

The nutrient media used for the bacterial growth were nutrient broth and nutrient agar. The nutrient media used for fungal growth were Tryptone soya broth and Potato dextrose agar. The media were prepared according to the manufacturer's instruction.

Preparation of Test Solutions

One gram of the extract was dissolved in 10ml of methanol to obtain a 10% test solution. From this, 5%, 2.5% and 1.25% test solutions were prepared through serial dilutions. Ampicillin (30 mg/ml) and Tiaconazole (10%w/w) were used as standards and methanol was used as the control.

Test for Antimicrobial Activity

Nutrient agar was poured into sterile petridishes. The plates were allowed to set and were incubated at 37^0C for 20minutes. Overnight culture of bacteria was poured on the dried sterile petridishes. The cork boner was used to cut holes at the centre . The holes were then filled with 10%, 5%, 2.5% and 1.25%

solution of the extract and Ampicillin and methanol The plates were then left at room temperature at 37^0C for 18 – 24hrs. After incubation, diameters of zones of inhibition were measured and recorded in millimeter

Test for Antifungal Activity

Potato dextrose agar was melted and cooled to about 45^OC and was then poured into clean, sterile petri-dishes and allowed to set. The overnight culture of the fungi was then poured on the sterile petri dishes. The plates were gently swirled round to enable the fungal suspension to cover the whole surface of the plates. A standard cork borer was used to cut six uniform and equidistant wells on the surface of the agar into which 10%, 5%, 2.5% and 1.25% solution of the extract and Tiaconazole and methanol were poured. The plates were then cultured at room temperature for 48hours. The diameters of zones of inhibition were measured and recorded.

Results

The phytotoxic activity of the saponins in lemon grass on the germination of maize and bean seeds and on the growth of the germinated seedlings showed that it is highly phytotoxic. The bean seeds were more sensitive to the saponin effect than the maize seeds because their growth were more relatively inhibited. The study also revealed that the inhibition of shoot and root lengths was concentration dependent. The higher the saponin concentration, the higher the inhibition of shoot or root length.

Table 1: Growth profile of seed culture treated with saponin extract from lemon grass.

Test Solution (%)	Maize		Beans	
	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)
Control	2.1	5.3	18.9	9.5
0.1	1.8	4.3	18.0	5.4
0.25	1.5	3.5	16.6	4.9
0.5	1.4	2.4	12.9	3.2
1.0	0.8	1.1	7.9	2.0

Table 2: Percentage Growth Profile Of Seed Cultures Treated With Saponin Extract From Lemon grass

Saponin Concn. (%)	Maize		Beans	
	Shoot (%)	Root (%)	Shoot (%)	Root (%)
0.1	85.71	81.13	95.20	56.84
0.25	71.43	66.04	87.80	51.58
0.5	66.67	46.28	68.25	33.68
1.0	38.09	20.75	41.80	21.05

The percentage growth was calculated using the following formula:

$$\text{Percentage growth} = \frac{GT}{GC} \times 100$$

GT = Average growth length of root or shoot in the test medium.
GC = Average growth length of root or shoot in the control medium

Table 3: Percentage growth inhibition of seedlings treated with saponin extracts from lemon grass.

Saponin Concn. (%)	Maize		Beans	
	Shoot (%)	Root (%)	Shoot (%)	Root (%)
0.1	14.29	18.87	4.80	43.16
0.25	28.57	33.96	12.20	48.42
0.5	33.33	54.72	31.75	66.32
1.0	61.91	79.25	58.20	78.92

The percentage growth inhibnition was calculated using the formula:
100 – (GT/GC x 100)

Antimicrobial tests showed that the saponins extract obtained from Lemongrass exhibited inhibitory activity against all the microbes selected. Two gram positive bacteria: *Staphylococcus aureus* and *Bacillus substilis*, two gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa* and two fungi: *Candida albicans* and *Aspergillus niger* were used in this study. The microbes were chosen because they are implicated in the etiology of human diseases (Hugo and Russel, 1983).

Staphylococcus aureus is a non sporing microorganism that causes skin lesion such as boils. Escherichia coil causes diarrhoea.

Candida albicans is a pathogen and is a constituent of the natural flora of mucus membranes of the respiratory, gastro-intestinal and female genital tract.

Aspergillus niger is one of the important causative agents of human mycotoxicoses and can easily contaminate stored food-stuff.

Table 4: Preliminary Antimicrobial Screening with 10% saponin solution

Microorganism	Type	Response
(1) Staphylococus aureus	Gram + ve	Inhibition
(2) Bacillus substilis	Gram + ve	Inhibition
(3) Escherichia coil	Gram – ve	Inhibition
(4) Pseudomonas aeruginosa	Gram – ve	Inhibition
(5) Candida albican	Yeast	Inhibition
(6) Aspergillus niger	Mould	Inhibition

Table 5: Inhibition of Microbial Growth By The Saponin Extract Of Lemon grass

Microorganisms	Control	Standard	1.25%	2.5%	5%	10%
S. aureus	0	26.0	12.0	14.0	17.0	19.0
E. coli	0	21.0	11.0	13.0	15.0	17.0
P. aeruginosa	0	18.0	0	10.0	12.0	14.0
B. substilis	0	23.0	0	11.0	13.0	15.0
C. albican	0	15.0	0	0	12.0	14.0
A niger	0	14.0	0	0	10.0	12.0

Table 6: The Percentage Inhibition Of Microbial Growth By Saponins In Lemon grass

Microorganism	1.25%	2.5%	5%	10%
S. aureus	46.2	53.9	65.4	73.1
E. coli	52.4	61.9	71.4	81.0
P. aeruginosa	-	55.6	66.7	77.8
B. substilis	-	47.9	56.5	65.2
C. albicans	-	-	80.0	93.3
A. niger	-	-	71.4	85.7

Percentage inhibition was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{DT}{DS} \times 100$$

DT = Diameter of inhibition by test solution
DS = Diameter of inhibition by standard antibiotic

Discussion

Phytotoxic evaluation of the saponin in lemon grass on maize and bean seed germination and on the growth of germinated seedlings showed that the saponin extract was significantly phytotoxic.

Inhibition of root and shoot lengths was concentration dependent and the shoots were less sensitive than the roots to saponin inhibition. The percentage growth inhibition of the root of maize and bean seedlings treated with the saponin at 0.25% were 33.96% and 48.42% respectively. The phytotoxic activity results from the inhibitory effect of saponins on plant growth hormones like Auxins and gibberellins (Waller, 1989, Waller 1993).

Phytotoxicity is a phenomenon which can be applied in agriculture and could be used in weed control and herbicidal application. This would make the saponin extract in lemon grass to be of immense importance in the allelochemical application for agricultural practices, although the leaves are used mainly as medicinal plant in traditional health delivery especially in the West African subregion.

The results obtained in this study showed that the saponins extract prepared from Lemon grass had pronounced antibacterial and antifungal effects. The saponins were highly effective against all the selected microbes even at extremely small concentrations of around 1.25%.

The antimicrobial activity of the saponin extract was tested on six pathogenic microbes namely *Staphylococcus aureus, Bacillus substilis, Escherichia coli, Pseudomonas aeruginosa, Candida albican*s and *Aspergillus niger*. The saponin extract inhibited the growth of all these microbes. At 10% dose concentration, the saponin extract was 73.1% and 81.0% as effective as Ampicillin (30 mg/ ml) inhibiting the growth of Staphylococcus aureus and *Escherichia coli* respectively.

The saponin extract has fungitoxic effects against *Candida albicans* and *Aspergillus niger*. At highest tested dose of 10%, the saponin extract was 93.3% effective against *Aspergillus niger* when compared with 10% Tiaconazole a standard antibiotic. This would make the saponins and of course the Lemon grass to be of immense importance in pharmaceutical and medical practice and seems to justify the use of Lemon grass in the traditional management and treatment of febrile illnesses especially of malaria fever in the South-Western zone in Nigeria.

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