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ANTIMICROBIAL ACTIVITIES OF HOT WATER EXTRACT AND PHYTOCHEMICAL SCREENING OF *ACALYPHA WILKESIANA* (RED ACALYPHA) ON SOME SELECTED ORGANISMS CAUSING INFECTION.

Salami Olufunmilayo Olamide[1], Afolabi Qasim Olaitan[2]

Microbiology Unit, Science Laboratory Technology Department of Federal College of Animal, Health and Production Technology Ibadan, Nigeria[1]
Chemistry Unit, Science Laboratory Technology Department of Federal College of Animal, Health and Production Technology Ibadan, Nigeria[2]

salamiolufunmi@yahoo.com, afolabiqasim@gmail.com

Abstract

Antimicrobial activities of hot water extract of Acalypha wilkesiana leaves growing in the premises of Federal College of Animal Health and Production Technology, Ibadan was carried out to verify claims of its medicinal properties. The extract was tested for its activity against Escherichia coli, Bacillus cereus, Staphylococcus aureus, Pseudomonas flourescens and Aspergillus sp. The extract exhibited activity against the organisms in varying degrees. In the agar diffusion test Aspergillus sp. and Bacillus cereus showed the highest zone of inhibition (50mm and 42mm) at the highest concentration of extract tested (100mg/ml) while Pseudomonas flourescens (35mm) , Escherichia coli (32mm) , and Staphylococcus aureus (27mm). Preliminary Phytochemical screening testing revealed the presence of Tannins, Saponin, Flavonoids, Alkaloids and Cardiac glycosides. It shows from the result that the Phytochemical present in the dry leaves extract is more compared to that of the fresh leaves quantitatively. Therefore the result gives scientific backing to the use of the leaves by the local people in the treatment of conditions usually associated with the organisms tested.

Keywords; Antimicrobial, Acalypha wilkesiana, Hot water, phytochemical test

INTRODUCTION

Acalypha wilkesiana is an evergreen shrub; it grows 3m high and spreads 2m across. The stem is erect with many branches, the branches have fine hairs with closely arranged crown, and the leaves are coppery green with red splashes of colour which gives them a malted appearance. It belongs to the family *Euphorbiaceace* and grows as an annual bedding plant (Oladunmoye, 2006). It is used to treat fungal skin diseases and was very effective in treating *Pityriasis versicolor*, *Tinea pedia*, and *Candida impetrigo* with 100% cure (Oyelami, 2003). Oyelami, 2003 further concluded that Acalypha wilkesiana ointment can be used to treat superficial mycoses.

The use of the plant, its extract or plant derived chemicals to treat diseases topical, subcutaneous and systemic has stood the test of time (Oladunmoye, 2006). In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs (Iniaghe *et al*, 2009). Also there has been little or no report of any form of microbial resistance during the use and administration of herbal medicines (Stephen *et al*, 2009). In West Africa, the water extract of Acalypha *wilkesiana* is traditionally used for treating skin problems and also as hedge plant (e.g. Ghana).

The leave and young shoot are used as vegetables, eaten with rice-dishes and popularly used for the treatment of gastrointestinal disorder and fungi infection particularly impetigo and tinea versicolor which affects the back, chest and axilla (armpit) of many babies in Nigeria. This study was done using *A. wilkesiana* leaves growing in school compound in Ibadan south western Nigeria and some bacteria and fungi which had hitherto not been tested in those previous studies were also tested.

MATERIALS AND METHODS

Plant preparation and Extraction

Leaves of *A. wilkesiana* free from pesticide were collected from FCAH and PT, Ibadan; the plant was identified at the botanical garden of Science Laboratory Department of the afore-mentioned College by a plant scientist. 150g of the leaves were washed with distilled water and crushed in pestle and mortar in the laboratory, the juice measured up to 100ml poured into conical flask with 700ml of absolute ethanol and some leaves was air dried and blended for which extraction was also done using cold extraction for Phytochemical screening. 200g of the powdered leaves was weighed on a metler balance and poured in a beaker and extracted with absolute ethanol (700ml) at room temperature. The extract was concentrated by recovering the solvent using water bath until the extract became just pourable at 40^0C.This was carried out to know the effectiveness of the extract when in dried form or juice form of the leaves.

Phytochemical screening

Testing for the presence of Phytochemical was carried out on the under listed sub-heading;

ALKALOIDS

About 0.5g of extract was stirred with 3ml of 1% aqueous hydrochloric acid on a steam bath and filtered; 1ml of the filtrate was treated with few drops of the following reagents;

1. Mayer's reagent
2. Picric acid solution
3. Dragendorff''s reagent

Presence of turbidity or yellow precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids.

TANNINS

0.5g of the extract was stirred with 1ml of distilled water and filtered. Ferric chloride ($FeCl_3$) solution was added to the filtrate. A blue-black, green or blue-green precipitate was taken as evidence for the presence of tannins.

STEROIDS (STEROIDAL RING)

0.1g of the extract was dissolved in 2ml of chloroform; sulphuric acid was carefully added to form a lower layer. A reddish brown color at the interphase is indicative of the presence of steroidal ring but in this case it is absent.

FLAVONOIDS

A 2g of powdered sample was detanned with acetone. The sample was placed on a hot water bath for all traces of acetone to evaporate. Boiled distilled water was added to the sample. The mixture was filtered while hot. The filtrate was cooled and 5ml of 20% sodium hydroxide was added to equal volume of the filtrate. A yellow solution is an indication of the presence of flavonoids.

GLYCOSIDES

A 0.1g of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. A 1ml of concentrated sulphuric acid was added gently by the side of the test tube. A reddish brown ring seen at the interphase and bluish green colour in the upper layer obtained indicates the presence of deoxy sugar characteristic of cardenolides.

Test Organisms

The test organisms were isolates from the Department of Science Laboratory Technology, Microbiology unit, Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State. The following organisms were used; *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Pseudomonas flourescens, Aspergillus sp.*

Preparation of the Test organisms

The isolates were sub-cultured onto differential solid media (MacConkey), peptone water and re-identified using various biochemical tests such as Catalase test, Citrate test, Motility test, Coagulase test, Oxidase test and Indole test.

RESULTS

Table 1; Antimicrobial activity of hot water extract of *Acalypha wilkesiana* leaves.

Isolates	Mean zone of inhibition diameter(mm)				
	100 g/ml	50 g/ml	25 g/ml	12.5g/ml	6.25g/ml
S. aureus	27	27	25	22	19
E. coli	32	31	20	30	30
B. cereus	42	42	37	37	26
Aspergillus sp	50	45	40	35	30
P. flourescens	35	32	32	32	28

From the table above, *Aspergillus sp* showed zone of inhibition of (50mm) at the highest concentration of extract tested (100g/ml) while *Bacillus cereus, Pseudomonas flourescens* and *Escherichia coli* showed zone of inhibition of 42, 35 and 32mm respectively. At extract concentration of

6.25g/ml, minimum zone of inhibition was observed on *Staphylococcus aureus* (19mm).

Table 2; **Disc Sensitivity Test (mm)**

Organisms	NIT	COT	AMX	TET	AUG	OFL	GEN	NAL
S. aureus	24	-	-	15	-	22	24	12
B. cereus	20	-	-	14	-	27	21	15
E. coli	17	-	-	-	-	12	16	-
P. flourescens	-	7	-	11	-	10	24	-
Aspergillus sp.	17	2	-	4	23	12	12	14

Keys:-NIT-Nitrofurantoin, COT-Cotrimozole, AMX-Amoxylin, TET- Tetracycline, AUG-Augmentin, OFL-Ofloxacin, GEN- Gentamicin, NAL-Nalixidic

Table 3; Comparism of antimicrobial activity of *A. wilkesiana* and disc sensitivity test.

		organisms			
	Antibiotics	*B. cereus*	*E. coli*	*P. flourescens*	*S. aureus*
Disc sensitivity test(mm)	NIT	20	17	-	24
	COT	-	-	7	-
	AMX	-	-	-	-
	TET	14	-	11	15
	AUG	-	-	-	-
	OFL	27	12	10	22
	GEN	21	16	24	24
	NAL	15	-	-	12
Antimicrobial activity of hot water extract of *A. wilkesiana*(mm)	100mg/ml	32	42	35	27
	50mg/ml	31	42	32	27
	25mg/ml	20	37	32	25
	12.5mg/ml	30	37	32	22
	6.25mg/ml	30	26	28	19

Table 2 shows different zones of inhibition of the antibiotics used against the organisms. Gentamicin shows the highest zone of inhibition on the entire selected organism e.g. *Staphylococcus aureus* and *Pseudomonas flourescens* compared to Ofloxacin and Nitrofurantoin which are active on *Staphylococcus aureus and Bacillus cereus*. It can be deduced that the plant extract is more effective on the organism unlike the antibiotics.

Table 4; Percentage yield of phytochemical present in the water extract and dry leaves of *Acalypha wilkesiana* respectively.

Components	Results	
	%Dry	%Fresh
Tannins	Present (3.97)	Present(1.68)
Flavonoids	Present (6.33)	Present(5.72)
Cyanide	Not Present (-)	Not present(-)
Glycosides	Present (3.25)	Present(1.56)
Alkaloids	Present (7.15)	Present(6.17)
Saponin	Present(14.77)	Present(6.91)
Steroids	Not present(-)	Not present(-)

Table 5; Morphological axis of isolated fungi

Organisms	Appearance	Colour	Spore Type	Nucleus
Aspergillus niger	Filamentous with branching mycelia	Surface is black, while the underneath is white to yellow	Long, smooth colourless or brown conidiospores	Haploid or Multinucle-ated
Aspergillus flavus	Filamentous with branching mycelia	Yellow-green, while the underneath is goldish to red-brown	Colourless, rough conidiospores	Multinucle-ated

Table 6; Confirmation of the identification of isolates

Organisms	Gram stain	Oxidase test	Catalase test	Coagulase test	Indole test	Motility test	Citrate test
E. coli	-	-	+	-	+	+	_
S. aureus	+	-	+	+	-	+	-
B. cereus	-	-	+	-	-	+	-
P. flourescens	-	+	-	-	-	+	-

Key (-) indicates negative, (+) indicates positive

DISCUSSION

The presence of zones of inhibition on the agar plates showed that the plant extract possesses antimicrobial activity on the tested organisms which included both Gram positive and Gram negative organisms. The zones of inhibition on the extract shown by *Aspergillus sp* and *Bacillus cereus* were higher than that exhibited by the standard drug when compared (Ofloxacin 27mm, Gentamicin 21mm,Nitrofurantoin 20mm). Also the ability of the extract to diffuse through the gel may be hindered because of large molecules (stearic hindrance) at higher concentrations of the extract, the zone of inhibition with the standard drug were comparable. Generally, the antimicrobial activity of the extract against *E.coli,and S.aureus* agrees with the earlier work of Oyelami,(2003) and Oladunmoye 2006. Phytochemical screening of the extract showed that the extract contains Glycosides, Flavonoids, Saponin, Alkaloids and Tannins.

Conclusion

Hot water extract of A. *wilkesiana* leaves growing in Ibadan,Nigeria was tested for its antimicrobial activity on some bacterial organisms in varying degrees. The research work showed that at 100g/ml (30 leaves), the leaf extract showed inhibitory activity against all the organisms tested especially *Bacillus cereus, Pseudomonas flourescens and Aspergillus sp*.

Recommendation

It is therefore recommended that at toxicological assay be carried out on the plant to ascertain its safety for drinking and further research be done to isolate the active ingredients responsible for the noted activities.

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