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Studies on phytochemical screening, antimicrobial and toxicity effect of the shoot system of *Acalypha segetalis* Müell. Arg.

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The dried and pulverized shoot system of *Acalypha segetalis* was extracted with hexane, ethyl acetate and methanol using cold extraction technique. The three extracts were subjected to phytochemical, toxicity and antibacterial screening. Phytochemical studies revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, glycosides, steroids and resins. The toxicity activity was investigated using brine shrimp (*Artemia salina*) lethality assay. The toxicity test showed that ethyl acetate and methanol extracts showed moderate toxicity with LC_{50} at 777.9711 and 221.8975 ppm respectively while hexane extract was non- toxic with LC_{50} 8252.5750 ppm. Antibacterial activity was tested employing the disk diffusion method revealed that the extract exhibited significant antibacterial activity against all the organisms used. Antimicrobial assay was concentration dependent; at higher concentrations such as 200, 100 and 50 mg/ml, the extracts exhibited strong inhibition and expectedly at lower concentrations such as 25 and 12.5 mg/ml, the activity of the plant extracts was low. The three extracts showed activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at all concentrations.

Key words: *Acalypha segetalis*, Euphorbiaceae, phytochemical, antimicrobial activity, brine shrimp lethality assay.

INTRODUCTION

Acalypha is a plant genus of the family *Euphorbiaceae*. The common names are copper leaves or three-seeded mercuries. These plants are mostly tropical or sub-tropical (but not present in Hawai and several other Pacific Islands); with a few representative in temperate zones. America contains two thirds of the known species, distributed from Southern United State to Uruguay and northern Argentina.

Acalypha segetalis is a shrub and eaten as a vegetable in some part of Africa (Burkill, 1994). In spite of the uses of members of *Euphorbiaceae* family from literature, little has been done on the chemical composition, toxicity of some species of *Acalypha*. Some common species are; *A. segetalis, Acalypha wilkesiana, Acalypha ciliata, Acalypha ornata, Acalypha hispidia*, *Acalypha indica*, *Acalypha fimbriata.* They belong to same kingdom, division, class, family and genus but different species (Aworinde et al., 2009).

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The essential oil of the leaves (Ogunwande et al., 2008) and whole plant (Aboaba et al., 2010) of *A. segetalis* have been studied. The brine shrimp toxicity assay of the essential oil revealed that it was toxic at LC_{50} 14.10 ppm. The secondary metabolites present in other species of *Acalypha* have also been the subject of discussion (Adesina et al., 2000; Bokshi et al., 2012; Rajaselvam et al., 2012). Flavonoids, alkaloids, phenolics, steroids, saponins, glycosides, phlobatanin were detected as some of the secondary metabolites in other *Acalypha* species. Different extracts of *A. wilkesiana*, *A. ornata*, *A. ciliata*, *A. indica*, *A. fimbriata* has been found to show moderate activities against different strains of bacteria and fungi (Gotep et al., 2010; Bokshi et al., 2012; Tehseen et al., 2012; Kasim et al., 2012) and also to *Artemia salina* and *anopheles gambiae* (Aboaba et al., 2012).

To the best of our knowledge, no record investigation has been reported on the phytochemical screening and isolation of the secondary metabolites in *A. segetalis*. The aim of this research is to determine the secondary metabolites in the extract of the shoot system of *A. segetalis,* the toxicity of the plant against brine shrimp larvae and to subject the extracts to antimicrobial screening.

MATERIALS AND METHODS

Plant collection and identification

Fresh plants of *A. segetalis* Muell. Arg. was collected from botanical garden of University of Ibadan, Ibadan. The plant was identified, authenticated and a copy was deposited at Forestry Research Institute of Nigeria (FRIN), herbarium section, Ibadan, Oyo State, Nigeria, with the herbarium number (109692) in November, 2013.

Plant extraction

The whole plant was separated from the root, air dried and pulverized the pulverized sample was weighed and kept for further analysis. The plant sample (600 g) was subjected to cold extraction by soaking in methanol for a week. The extract was concentrated using rotary evaporator at 35°C. The crude extract was then partitioned in n-hexane, ethyl acetate and methanol. Each extract; n-hexane, ethyl acetate and methanol were concentrated separately using rotary evaporator at 35°C.

Phytochemical screening

The extracts obtained above were screened for alkaloids, flavonoids, steroids, saponins, phenols, tannins, glycosides and resins using standard methods as described by Harborne (1998).

Toxicity analysis

Brine shrimp lethality test for toxicity

The brine shrimp lethality assay was used to test for the toxicity of the extracts. Brine shrimp eggs were hatched in sea water and allowed to stay for 48 h at room temperature. The nauplii (harvested shrimp) were attracted to one side of the vials with a light source. Solutions of the extracts were made in DMSO at varying concentration (1000, 100 and 10 µg/ml) and incubated in triplicate test tubes with brine shrimp larvae. Ten brine shrimp larvae were placed in each of the triplicate test tubes. Control brine shrimp larvae were placed in a mixture of sea water and DMSO only. After 24 h, the number of larvae that survived in each test was determined. The concentration at 50% mortality of the larvae (LC_{50}) was determined using the Finney computer programme (Meyer et al., 1982; McLaughlin et al., 1998).

Preparation of sample for antimicrobial analysis

1 g of each sample was weighed and dissolved in 5 ml of the solvent used for the extraction to give 200 mg/ml. This was serially diluted to obtain lower concentrations of 50, 25 and 12.5 mg/ml. Gentamycin (5 mg/ml) was used as the positive control for bacteria while griseolvuvin (70%) for fungi and the negative control contained the solvent used for dissolving each extract.

Agar diffusion: pour plate method for bacterial

Cultures of each of the organisms used were prepared. The organisms were *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella Pneumonia* and *Salmonella typhi* were prepared. A sterile cork-borer was used to create wells (or holes) inside the set plates. The test solutions of oils (50 µl) at concentration of 40 mg/ml were then introduced into each of the designated cups on each plate ensuring that no spillage occurred. The same amount of the standard antimicrobial agent and solvents were introduced using syringes into the remaining cups on each plate to serve as positive and negative controls respectively. The plates were left at room temperature for 2 h, allowed to diffuse into the medium, turned upside-down and thereafter incubated at 37°C for 24 h in an incubator. Clear zones of inhibition were observed. Activity or inactivity of each extract was tested in triplicate and the diameters of zones of inhibition were measured in millimeter (mm) using a transparent well-calibrated ruler. The positive control for bacteria is gentamicin at the concentration of 5 mg/ml. The analysis was done in triplicates (Belboukhari and Cheriti, 2005).

Agar diffusion plate method for fungi

Sterile Sabouraud Dextrose Agar (SDA) was poured aseptically into the sterile plates and allowed to cool down for 45 min. 0.2 ml of 1:100 dilutions of the organisms *Candida albicans, Rhizopus stolonifer, Aspergillus niger* and *Penicillum nonatum* were spread on the surface using a sterile spreader. Then, a sterile cork-borer was used to create wells inside the plates. The same procedure described for anti-bacterial activity above was followed from this stage. The positive control for the fungi was 70% griseolvuvin. All the plates for the fungi were incubated at 28°C for 48 h. The clear zones of inhibition were observed and recorded using the same method as described in the case of bacteria (Andrew, 2001; Thongson et al., 2004).

RESULTS

The yields of the methanol, ethylacetate and hexane extracts were 2.25, 1.33 and 1.19% respectively. The

Table 1. Phytochemical screening of shoot system of *A. segetalis*.

Secondary metabolites	ASH	ASE	ASM
Alkaloids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Sterols	-	+	+
Flavonoid	-	+	+
Resins	+	+	+
Phenols	-	+	-
Glycosides	-	+	+

ASH =*Acalypha segetalis* hexane extract, ASE =*Acalypha segetalis* ethyl acetate extract, ASM = *A. segetalis* methanol extract + = positive, - = negative.

Table 2. Brine shrimp toxicity test.

Extract	LC_{50} (ppm)	G
Hexane	8252.58	0.5927
Ethyl acetate	221.90	0.2957
Methanol	777.997	0.2327

"LC_{50}", is the lethality concentration 50%, "G", is the degree of freedom.

percentage moisture content of the shoot system sample of *A. segetalis* was 13.57% this implies that the moisture content loss in the shrub is minimal. The result of phytochemical screening in Table 1 showed that methanol extract are rich in alkaloids, saponins, resins, tannins, sterols, glycosides and flavonoids while it gave a negative result for phenol. The ethylacetate extract contain alkaloids, tannins, saponins, sterols, flavonoids, resin, phenols, glycosides while the hexane extract gave positive results for alkaloids, tannins, saponins and resin but no sterols, flavonoids, resin, phenols and glycoside.

Toxicity of the extracts against brine shrimps show LC_{50} of 777.9711, 221.8975 and 8252.5750 ppm for methanol, ethylacetate and hexane extracts respectively (Table 2). The antimicrobial assay of the three extracts showed that at high concentrations; 200 and 100 mg/ml, the three extracts were active against the ten micro-organisms used but lower than the positive control. At 200 mg/mL, 100, 50 and 25 mg/ml, methanol extract was active against *S. aureus, B. subtilis, S. typhi, K. pneumonia, C. albican, A. niger, R. solonifer* and *P. notatum* but at 12.5 mg/ml, it displayed low activity against *E. coli, B. subtilis, C. albican* and *A. niger*. For ethyl acetate extract, it was active against all organisms tested for at 200, 100 and 50 mg/ml, but not active against *E. coli, S. typhi, K. pneumonia, C. albican and R. stolonifer.* However at 12.5 mg/L, no zone of inhibition was observed against *P. notatum*. For the hexane extract, at 200, 100, 50 and 25 mg/ml it was active against all organisms tested for, but at 12.5 mg/ml it was not active against *S. aureus, E. coli, B. subtilis, S. typhi, A. albican, A. niger* and *P. notatum*. Comparatively, at 12.5 mg/ml being the lowest concentration, there was no zone of inhibition shown against *E. coli, C. albican and A. niger* for the three extracts. Zone of inhibition decreases from the highest concentration 200 mg/ml to the lowest concentration 12.5 mg/ml. Ethyl acetate and methanol extracts have the highest zone of inhibition against *P. aeruginosa*, being 28 mm and 24 mm respectively. At the lowest concentration of 12.5 mg/ml, hexane extract was active against *P. aeruginosa, K. pneumonia, R. stolonifer* as shown in Table 3.

DISCUSSION

The yields of the three extracts under study that is; methanol, ethylacetate and hexane gave an expected range considering the polarity of the three solvents. Expectedly, methanol the most polar, ethylacetate moderately polar while hexane a non-polar solvent and as such methanol gave the highest yield followed by ethylacetate and then hexane. The phytochemical from this report is comparable with another species of *Acalypha* previously reported (Adesina et al*.,* 2000). These classes of compounds present from the phytochemical screening are responsible for the curative activity against several pathogens and therefore could justify the ethno botanical uses of the plant for treatment of a wide range of illness. For example, glycosides,

Table 3. Antimicrobial Activity of *Acalypha segetalis* extracts.

Extract	Conc. (mg/ml)	*S. aureus*	*E. coli*	*B. subtilis*	*P. aeruginosa*	*S. typhi*	*K. pneumonia*	*C. albicans*	*A. niger*	*R. solonifer*	*P. notatum*
Methanol	200	20	20	18	28	18	20	18	16	20	20
	100	18	18	14	20	16	18	16	14	18	16
	50	16	14	12	18	14	14	14	12	16	14
	25	12	10	10	14	12	12	10	10	14	12
	12.5	10	-	-	10	10	10	-	-	10	16
Ethylacetate	200	24	18	20	28	18	18	16	14	18	16
	100	20	16	16	26	16	14	14	12	14	14
	50	18	14	14	20	12	12	12	10	12	10
	25	14	10	12	14	16	10	10	-	10	-
	12.5	10	-	10	10	-	-	-	-	-	-
Hexane	200	20	18	16	24	20	18	16	18	20	18
	100	18	14	14	20	16	16	14	14	16	14
	50	14	12	12	16	14	14	12	12	14	12
	25	12	16	16	12	10	12	10	10	12	10
	12.5	-	-	-	10	-	10	-	-	10	-
Controls	-ve	-	-	-	-	-	-	-	-	-	-
	+ve	38	36	34	40	36	34	24	26	28	26

S. aureus = *Staphylococcus aureus.* (Bacteria), *E. coli* = *Escherichia coli.* (Bacteria), *B. Subtilis* = *Bacillus subtilis.* (Bacteria), *P. Aeruginosa* = *Pseudomonas aeruginosa.* (Bacteria), *S. Typhi* = *Salmonella typhi.* (Bacteria), *K. pneumonia* = *Klebsiellae pneumonia.* (Bacteria), *C. albican* = *Candida albicans.* (Fungi), *A. niger* = *Aspergillus niger.* (Fungi), *R. stolonifer* = *Rhizopus stolonifer* (Fungi), *P. notatum* = *Penicillum notatum.* (Fungi), -ve: Negative controls; Methanol for methanol extract, ethylacetate for ethylacetate extract and hexane for hexane extract, +ve: Positive control; Gentamicin 5 g/ml; bacteria and Griseolvuvin 70%; fungi.

saponins, flavonoids, tannins and alkaloids have hypoglycemic activites, anti-inflammatory activities (Augusti and Cherian, 2008), sterols, triterpenoids and saponnins have also been reported to show analgesic properties and central nervous system activites (Argal and Pathak, 2006). Plants having alkaloids are used in medicines for reducing headaches and fever; these are attributed for antibacterial and analgesic properties (Pietta, 2000).

The results of the brine shrimp lethality test was interpreted according to Moshi et al. (2014); extracts with $LC_{50} \leq 1000$ ppm is toxic while extracts with $LC_{50} \geq 1000$ ppm. The LC_{50} of the methanol, ethyl acetate fraction and hexane fraction gave 777.9711, 221.8975 and 8252.5750 ppm respectively. This shows that hexane fraction is non toxic, while ethyl acetate and methanol fraction are regarded as moderate level of toxicity and very low toxicity respectively. In a related study, *A. hispida* showed displayed a very high level of toxicity (LC_{50} 7 ppm) even when compared to a standard drug chloramphenicol (Bokshi et al*.,* 2012). Also the essential oils from *A. ciliata* and *A. ornata* plants revealed various larvicidal activities causing 100% mortality against *Artemia salina* and *A. gambiae* respectively. The LC_{50} values of *A. ornata* and *A.ciliata* oils estimated against *A. gambiae* s.l. were 77.59 and 73.96 ppm respectively (Aboaba et al., 2012).

Plant extracts contain different phytochemical with biological activity that can have valuable healing properties. The demonstration of broad spectrum of antimicrobial activity by *A. segetalis* may help to discover new antibiotic substances that could serve as agents for the control of infectious diseases. It was observed that methanol extracts gave the highest activity against the tested organisms compared to the ethylacetate and hexane extracts, the rational for this observation may be based on the ability of polar extracts permeability through the plasma membrane. The methanol extract showed inhibition against all the organisms used at all the tested concentrations except at the lowest which is 12.5 mg/ml where it displayed low activity against *E. coli, B. subtilis, C. albican and A. niger*. Ethyl acetate extract was active against all organisms tested for at 200, 100 and 50 mg/ml, but not active against *E. coli, S. typhi, K. pneumonia, C. albican and R. stolonifer* while the hexane extract was active against all organisms tested for at 200, 100, 50 and 25 mg/ml, but at 12.5 mg/ml it was not active against *S. aureus, E. coli, B. subtilis, S. typhi, A. albican, A. niger* and *P. notatum*.

Conclusion

Phytochemical analysis of plants is very important as it could explain the use of these plants traditionally for the treatment of some ailments. The extracts of *A. segetalis* from this study contain secondary metabolites like alkaloids, tannins, saponins, sterols, flavonoids, resin, phenols, glycosides which play a vital role in preventing various diseases. The antimicrobial assay results indicate that the plant possesses considerable antimicrobial activity that supports the traditional uses of this plant and other species of *Acalypha* in the treatment of various diseases. However, advance studies are required to identify and characterize the chemical compounds, that is, structures of the secondary metabolites responsible for these observed activities.

Conflict of Interest

The authors have not declared any conflict of interest.

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