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Insecticidal, antimicrobial, phyto- and cytotoxicity of C*hassalia kolly* plant extract

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ABSTRACT

Chassalia kolly (Schumach.) Hepper (Rubiaceae) is used in ethno-medical practices particularly in Nigeria and the West African sub-region for the treatment of typhoid and fevers that are endemic in Nigeria as well as in the tropics and as an insect repellant. The methanolic extract of the dried whole plant Chassalia kolly was investigated for insecticidal, antimicrobial, phyto- and cytotoxicity activities. Antifungal activity was observed against only one of the three strains investigated namely, Candida albicans The extract however inhibited the growth of all the five human pathogenic bacteria namely: Salmonella typhii, Escherica coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus tested in the agar cup plate diffusion technique, exhibiting a concentration dependent activity. Cytotoxicity was evaluated using brine shrimp lethality assay revealing its relatively non-toxicity with an LD_{50} value greater than 1000 µg/ml. Phytotoxicity using the Lemna bioassay showed a moderate growth inhibitory effect against Lemna minor. The insecticidal assay by contact toxicity method also revealed a moderate insecticidal rate of 40% against Rhizopertha dominica at the concentration of 1572.7 µg/cm^2.The extract contains glycosides, alkaloids and flavonoids. These results provide some scientific basis for the utilization of the plant in ethno-medicine for the treatment of typhoid fever and as an insect repellant.

Key words: *Chassalia kolly*, antimicrobial, insecticidal, phyto-and cytotoxicity.

INTRODUCTION

Chassalia kolly (Schumach.) Hepper (Rubiaceae) is a soft-stemmed shrub native to West Africa [1]. There is very little information in literature about the medicinal properties of this plant, however oral reports from herbal medical practitioners indicate that the extract of this plant is

usually given to patients to drink for the treatment of typhoid fever and fevers endemic in Nigeria as well as in the West African sub-region. The plant family Rubiaceae is a well known source of numerous natural products. Rubiaceae plants are noted for their rich source of alkaloids especially the cinchona alkaloids of which the active antimalarial constituent quinine was first isolated [2]. In addition some other constituents from Rubiaceae plants have been found to possess antiamoeboid, antileprotic, antiparalytic, diuretic and antibilharzia properties. Other classes of natural products such as indole, oxindole, quinoline and purine type alkaloids, anthocyanins, anthraquinones and iridoid glycosides have been known to occur in a number of species of Rubiaceae. Plant containing stimulants such as coffee from *Coffee arabina*, hallucinogenic and analgesic preparations from *Psychotoria* species are also a regular feature of the family [1,3].

Chassalia species have been prescribed for wound dressing, inflammation and liver disease and as an insect repellant. Novel HIV inhibitory macrocyclic peptides have been isolated from the tropical tree *Chassalia parvifolia* [4,5]. In continuation of our studies on biological activities of medicinal plants and search for source of new therapeutic drugs [6-8], we now report on insecticidal, antimicrobial, phyto- and cytotoxicity of *Chassalia kolly* whole plant extract.

MATERIALS AND METHODS

Collection, Authentication and Extraction of Plant Material

The plant (*Chassalia kolly*) used for this study was collected within Ibadan town and identified by Mr. Felix Usang of Forest Research Institute of Nigeria (FRIN) where a voucher specimen (FHI107662) was deposited. The air dried whole plant (1kg) was extracted with methanol for 48 hours and the resulting plant extract (15 g) was stored in the refrigerator at 4^{o}C prior to use. Preliminary phytochemical screening was carried out on the extract using standard screening methods [9].

Phytochemical screening

Preliminary Phytochemical screening for various secondary metabolites such as anthraquinones, tannins, cardiac glycosides, alkaloids, saponin glycosides and the steroidal nucleus were carried out for the methanolic whole plant extract using the usual procedures [9].

Antimicrobial Assay

Microorganisms: Cultures of five human pathogenic bacteria were used for the in vitro antibacterial assay while three fungi were utilized . All the microorganisms (namely: *Salmonella typhii*, *Escherica coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albican, Asperigillus niger* and *Dermatophyte sp.*) were obtained from the laboratory stock of the Department of Microbiology, University of Ibadan, Ibadan.

Media: Nutrient broth, nutrient agar, sabourand dextrose agar (SDA), tryptone soya agar (Oxford Laboratories, U.K.) were used in the assays. Methanol (Merck) was also used in solublising the extracts \ drugs and as a negative control in the assays.

Antimicrobial agents: Ampicillin, 12.5µg/ml (Lab Oftalmiso, Spain), tioconazole cream 12.5µg/ml (Pfizer Inc., New York) were included as standard reference drugs in the study.

Antimicrobial activity determination: The cup agar broth diffusion procedure [10] was used. An overnight broth culture of 1-2 x 10^7 CFU of each bacterium was used to seed sterile molten agar medium maintained at 45^o C. Sterile tryptone soya agar plate was similarly seeded with fungi. Five wells (10mm) respectively, were bored in each plate (7mm, diameter) with an aseptic cork borer when seeded plates had solidified. 200mg/ml of extract was reconstituted in methanol and 80µl dispensed into each of the wells with the aid of a Pastuer pipette. Diameters of zones of inhibition were determined after incubating plates at 37^o C for 24 hr (bacteria) and at 25^o C for 72h (fungi). When seeded with bacteria, each plate had wells filled with methanol as well as ampicillin and for fungi, tioconazole was filled in one of the wells also. This method is similar to previous procedures [11].
Antimicrobial studies were done in triplicates and diameters of zones of inhibition (mm) are expressed as the mean and standard errors on means. Student's "T" test was used to test probability at $P < 0.05$.

Table 1: Antimicrobial activity of Methanol Extract of *Chassalia kolly*

Plant Extract	Conc[a] (mg/ml)	Mean Diameters of zones of Inhibition of bacteria in mm (+SEM[b])					Mean Diameters of zones of Inhibition of fungi in mm (+SEM[b])			
		B. subtilis	*S. typhii*	*S. aureus*	*E. coli*	*P. aeruginosa*	Conc[a] (mg/ml)	*A. niger*	*C. albicans*	*D .spp*
Whole Plant	20	12±0.1	10±0.2	10±0.6	-	-	20	-	-	-
	40	16±0.1	15±0.3	12±0.3	10±0.3	9±0.5	40	-	-	-
	70	20±0.2	20±0.1	14±0.3	12±0.1	12±0.6	70	-	-	-
	100	22±0.1	20±0.4	14±0.2	15±0.3	16±0.2	100	-	15±0.1	-
	Amp	20±0.5	20±0.2	15±0.1	25±0.4	12±0.3	Tioconazole	22±0.2	20±0.5	12±0.2

[a] *Methanol was used for dissolving the extracts as well as the control*
[b] *N=3*

Phytotoxicity
The Lemna bioassay was carried out using the modified protocol of Mclaughlin [12,13]. The *Lemna minor* (Duckweed) were cultivated under optimum conditions for 1 to 2 days, briefly washed in water and transferred into the E-medium nutrient (a mixture of various constituents adjusted to pH 5.5-7 to provide nutrients for growth of plant) prior to use. The flasks used for the bioassay were initially inoculated with 10, 100 and 1000 µL in each of three replicates of the stock solution of the extracts (30 mg of crude dissolved in 1.5 mL MeOH / EtOH). The solvents were left to evaporate overnight, thus yielding 10, 100 and 1000 μgml^{-1} medium flasks to which 20ml of E-medium and 10 plants of *L. minor*- each containing a rosette of 2-3 fronds was introduced. Other flasks containing solvent and reference/standard drug paraquate served as negative and positive controls, respectively.

The flasks were placed in growth cabinets maintained at 28 ± 1^oC for 7 days and examined daily during incubation. The number of fronds per flask was counted on day 7 to determine the growth inhibition or proliferation of fronds in the flasks. The percentage growth regulation was therefore analysed with reference to the negative control [14].

Cytotoxicity
The eggs of brine shrimp *Artemia salina* were readily available as fish food in pet shops. The eggs hatched within 48 hours of being placed in artificial sea water.

The Brine shrimp lethality assay on the extracts were carried out using initial concentrations of 10, 100 and 1000 μgml^{-1} in vials containing 5ml of brine and ten shrimps (*Artemia salina*) in each of three replicates using the modified method of Mc laughlin [12,13]. Survivors were counted after 24 hrs. The data were processed using a Finney computer programme and LD_{50} values were obtained. Solvent and the reference cytotoxic drug (Etoposide) served as negative and positive controls respectively.

Insecticidal activity
Test Insect. *Rhizopertha dominica* (with a lifecycle of 30 days) were reared from wheat and gram seeds under controlled conditions (at 30^oC, relative humidity of 50-70%) in plastic bottles containing sterile breeding media. Insects of uniform age and size were used for the assay.

Sample Preparation. Concentrations of 393.17 $\mu g/cm^2$ in volatile organic solvent (ethanol) of standard insecticide drug permethrin and 1572.7 $\mu g/cm^2$ of the crude extract were used for the assay. Petri plates containing filter papers treated with ethanol and reference/standard drug paraquate served as negative and positive controls, respectively.

Insecticidal Activity Determination. Contact toxicity using the method of impregnated filter paper test was employed [15,16].
Day 1. Filter papers cut to size of Petri plates (9cm) were placed in the plates and the prepared samples were loaded over the filter paper with the aid of 1000µl micropipette and left for 24 hours for the solvent to evaporate completely.

Day 2. 10 healthy and active insects of same size and age were placed in each plate (test and controls) the next day with the help of clean brushes. The plates were incubated at 27^oC for 24 hours with 50% relative humidity in the growth chamber.

Day 3. Survival of the insects were assessed by counting the number of survivals and the Percentage Inhibition or Mortality were calculated [17]. Tests were done in triplicate.

RESULTS AND DISCUSSION

The results of the phytochemical screening revealed the presence of glycosides, alkaloids and flavonoids in the extract. Phytotoxicity using the Lemna bioassay revealed a moderate growth inhibitory effect against *Lemna minor*. Cytotoxicity was evaluated using brine shrimp lethality assay. The extract was found to be relatively non-toxic as it had an LD_{50} value greater than 1000 µg/ml. The insecticidal assay by contact toxicity method also revealed a moderate insecticidal rate of 40% against *Rhizopertha dominica* at the concentration of 1572.7 $\mu g/cm^2$. Diameters of zones of inhibition ≥ 10mm were considered active [10]. Antimicrobial studies were done in triplicates and diameters of zones of inhibition (mm) are expressed as the mean and standard errors on means (SEM). Student's "T" test was used to test probability at $P < 0.05$. Results of the antimicrobial assay are as shown in Table 1. The extract did not exhibit antifungal activity except

at high concentration against *Candida albicans.* The extract however, inhibited the growth of all the bacteria tested in the agar cup plate diffusion technique, at concentrations ranging from 20 to 100mg/ml, exhibiting a concentration dependent activity. The fact that the extract produced inhibitory activities comparable to the reference drug ampicillin on four of the microbes including *Salmonella typhii*, is indicative of highly promising antibacterial constituents especially of antityphoid activity for which this plant is used in folklore medicine.

CONCLUSION

To the best of our knowledge there is no earlier report on the insecticidal, antimicrobial, phyto- and cytotoxicity of the plant. The results of this study indicate that the extract is relatively non toxic, antibacterial, mildly insecticidal and phytotoxic in action. Further investigations are currently on to isolate the natural constituents responsible for the observed activity in the extract and these will be reported at a later date.

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