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Research Paper

Chrysophyllum albidum seed (African star apple) as an additive in agricultural feed and a potent antimicrobial

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This study was aimed at determining the usefulness of *Chrysophyllum albidum* seed cotyledon as an animal feed additive and the antimicrobial potencies of the seed extracts. Wister Rats, were fed with different percentages of the *C. albidum* seed mixed with commercial animal feed showed no significant change in their hematological parameters and the histology of the Wister rats' vital organs. The hematological parameters studied include Part Cell Volume, White Blood Cell, Red Blood Cell, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration and differential white blood cell concentration. The antibacterial effect was determined using agar well diffusion assay. Phytochemical analysis of the ethanol extract of *C. albidum* indicated the presence of flavonoids, tannins, phenols, steroids and saponins. The seed had no adverse effect on the hematological indices of the rat. The maximum activity of ethanol extract was observed on *Staphylococcus aureus*, which showed clear zones of diameter of 13.0 mm at a Minimum inhibitory Concentration value of 62.5 mg/ml while it had low activity on *Pseudomonas aeruginosa*, with clear zone of inhibition of 9.0 mm at the same MIC value. The extract, at all concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml), showed potent antibacterial activity. The ethanolic extract showed better inhibition against *Staphylococcus aureus* (ranged from 500 mg/ml to 31.3 mg/ml*), P. aeruginosa* and *E. coli* (ranged from 500 mg/ml to 62.5 mg/ml). The Minimum bactericidal concentration of the extract against *E. coli* was at 500 mg/ml and 250 mg/ml, *S. aureus* (ranged from 500 mg/ml to 125 mg/ml, and 500 mg/ml) against *P. aeruginosa.* The potent antibacterial effect of the extract could be attributed to the wide range of active metabolites. Therefore, the seed of *C. albidum* can be a good source of remedy in phytomedicine and agricultural use.

Keywords*: C. albidum,* antimicrobial, animal feed, histology, hematology

INTRODUCTION

African star apple (*C. albidum)* is a dominant canopy tree of lowland and mixed rainforests, sometimes, riverine (Madubuike and Ogbonnaya, 2003). The plant is propagated by seedlings, wildings and direct sowing. It is a seasonal fruits-bearing tree. The fruit is fleshy and juicy, producing whitish gummy exudates (Adewusi, 1997). It is widely eaten in South Western Nigeria, popular among women and children (Amusa *et al.,* 2003). The fruit pulp is rich in ascorbic acid and suitable for jams. The fruits can be fermented and distilled for the production of wine and spirits in alcohol making. It is a potential source of soft drinks (Bello and Henry, 2015).

The seeds of *C. albidum* are about 1-1.5 x 2 cm, beanlike, shiny when ripe, compressed, with one sharp edge and a star-shaped arrangement in the fruits (Orwa *et al.,* 2009). The seed coats hard, bony, shiny, and dark brown, and when broken reveal white-colored cotyledons (Amusa *et al.,* 2003). There are several studies carried out on different aspects of African Star Apple. Examples include the effects of ethanol bark extract on biochemical and hematological parameters (Adebayo *et al.,* 2010). Also, study published in the Journal of physiology and pathophysiology by Adeoye *et al.* (2012) suggested that the methanol bark extract may provide the next best antimalarial drug. *C. albidum* is good for treatment of fibroids (Faleyimu and Oluwalana, 2008). Its content of

natural antioxidants have been established to promote health by acting against oxidative stress related disease such as diabetes, cancer and coronary heart diseases (Orijajogun *et al.,* 2013).

The fruits of *C. albidum* have immense economic potential, especially following the reports that jams that could compete with raspberry jams and jellies could be made from it (Adisa, 2000). The fleshy fruits pulp is suitable for jams and is eaten especially as snack by both young and old (Amusa *et al*., 2003). The fruits have been found to have highest content of ascorbic acid per 100 g of edible fruits or about 100 times that of oranges and 10 times that of guava or cashew (Idowu, 2006). It is also reported that it is an excellent source of vitamins, irons, flavors to diets (Adisa, 2000). In addition, its seeds are a source of oils, which is used for diverse purposes (Amusa *et al.,* 2003). The potentials of the seeds of *c. albidum* have not been fully utilize and during its season they are found as litters around communities constituting environmental pollution (Adewusi, 1997) (Figure 1). This work is aimed at investigating the potentials of the seeds as additives in animal feeds as well as the antimicrobial potency.

Fig. 1A: Chrysophyllum albidum Fruit

Fig. 1B: Chrysophyllum albidum seed (With Shell)

Fig. 1C: Chrysophyllum albidum seed (Dried Without Shell)

MATERIALS AND METHODS

Sample collection

The African star apple seeds cotyledons were collected in Ibadan, Oyo State, Nigeria. The seeds were identified and authenticated at the Herbarium of the Department of Biological Sciences, University of Abuja, Abuja, Nigeria.

Experimental animals

Eight Albino Wister rats (6 females and 2 males) with weights ranging from 111 g – 133 g obtained from the Veterinary Medicine Department of University of Abuja, were used for the experiment.

Media used is from hi-media laboratories limited, India

Nutrient agar, Mueller Hinton Agar, Sabouraud Dextrose Agar, Nutrient broth, Peptone water broth, Urea agar, Simmon Citrate agar.

Chemicals and reagents

Crystal violets, Safranin, Lugols iodine, Hydrogen peroxide, Hydrochloric acid, Chloroform, Ferric acid, Sulphuric Acid, Kovacs reagent, Ethanol, Acetone from Sigma Aldrich were used.

METHODS

Preparation of sample

The sample was prepared according to the methods described by Anibijuwon and Udeze (2009). The seeds of *C. albidum* were air dried to constant weight. The dried seeds were pounded into smaller granules using laboratory Mortar and pestle and then grinded with a blender. The grinded sample was then kept in an air tight container.

Feeding of rats

The Wister rats were maintained in 12 h light and 12 h darkness and kept in the animal house of the Department of Biological Science, University of Abuja, Abuja, Nigeria. The animals were acclimatized for two weeks. The animals were all treated in accordance with recommendations of National Institute of Health (NIH) Guidelines for the care and use of laboratory Animals (NIH, 1985). After completion of the Acclimatization period, the rats were divided into three (3) groups with a control group, their weights were checked and they were fed with 20 grams daily (varying percentage of grinded seed cotyledons of *C. albidum* and commercial animal feed). The grouping of the rats is as follow:

Control ----------One female and one male
Group A----------One female and one male
Group B----------Two females
Group C----------Two Females

The rats were dissected after administration period and their vital organs (spleen, liver, kidney and brain) were examined physically for any noticeable changes to know the effects of the *C. albidum* seed cotyledons on the rats. Percentage of feed given is as follows:

Group A = 20 g of grinded raw seed (100%)
Group B= 10 g of commercial animal feed + 10 g of seed (50% + 50%)
Group C = 4 g of seed + 16 g of commercial animal feed (20% + 80%)
Control = 20 g of raw feed (100% feed)

Collection and hematology of blood sample which includes; WBC, PCV, RBC, MCH, MCHC

Blood samples were collected from the rats into EDTA bottles. Hematology tests (Full Blood Count) was carried out on the blood samples.

Ethanol extraction of the seed

The method described by Arekemase *et al.* (2011) was used for ethanol extraction of the seed cotyledons. 100 g of finely grounded seed cotyledons was extracted by cold extraction in 500 ml of ethanol. The mixture was soaked for 72 h, the resulting mixture was then filtered using Whatmans filter paper No. 1 and a Rotary evaporator was used to obtain the filtrate. The extract was weighed and stored in a sterile bottle.

Phytochemical screening

Phytochemical screenings was carried out on the seed cotyledons of *C. albidum* to determine the phytochemical constituents such as Test for Alkaloids, flavanoids, terpenoids, tannins, phenol, steroids and saponins as described by Akinyemi *et al.* (2005); Junaid *et al.* (2006) and Prashant *et al.* (2011).

Antimicrobial screening

Pathogenic clinical isolates of *E. coli, S. aureus* and *P. aeruginosa* were collected from Ritchez medical laboratory, Maitama, Abuja, Nigeria. The isolates were authenticated in the Microbiology laboratory of the Department of Microbiology University of Abuja by sub culturing on selective and differential media (Arekemase *et al.,* 2011).

Preparation and sterilization of media

Media employed in this study were weighed appropriately and prepared according to the manufacturers' instruction and sterilized in the autoclave at 121°C for 15 min. After sterilization, the media were allowed to cool before aseptically dispensing into petri dishes and then allowed to solidify.

Biochemical tests

Biochemical tests including; Gram Stain, Catalase test, Indole test, Urease test and citrate test were carried out on each of the pure isolates (Cheesbrough, 2002; Baker *et al.,* 1998). The isolates were maintained in an Agar Slant in the refrigerator set at 4°C.

Preparation of test organisms

The clinical isolates were maintained on agar slant in bijou bottles and then sub-cultured for 24 hours before use. Pure isolates of each test organisms were emulsified into 5 ml of sterile normal saline using a sterile bacteriological loop to make a suspension of the test organism. The suspension was standardized to match 0.5 McFarland standards, shaken vigorously to ensure thorough mixing and stored overnight Arekemase *et al.* (2011).

Reconstitution of extracts

Reconstitution of the extract was done according to the method described by Arekemase *et al.* (2011). This was done by diluting the each seed extract (500 mg, 250 mg, 125 mg and 62.5 mg) in 1 ml of Dimethyl sulphuroxide (DMSO) to give different concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml, respectively.

Determination of the antimicrobial activity of the seed extract

The antimicrobial activity of the seed extracts was determined using the agar well diffusion method described by NCCLS (1990) preparing concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml. The Control experiments were done using 500 mg/ml of Chloramphenicol. The inhibition zone was measured with a transparent meter rule and the sensitivity test of the seed extracts was done in duplicates.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the seed extract was determined by using agar dilution to various concentrations to the macro broth dilution technique (Baron and finegold, 1990; Akinyemi *et al.,* 2005). The lowest concentration of the extract that prevented the growth of the tested organism is the MIC.

Minimum bactericidal concentration

The minimum bactericidal concentration (MBC) was determined following the procedure as described by

Akinyemi *et al.*(2005).

Statistical analysis of variance

The mean values of rats weights obtained before and after treatment with the seed cotyledon and the mean zones of inhibition values obtained for the antimicrobial activities of the seed extract of *Chrysophyllum albidum* activities on the test bacteria were subjected to one – way Analysis of Variance (ANOVA) at p = 0.05 level of significance.

RESULTS

The effects of *Chrysophyllum albidum* seed on weights of the treated Wister rats is shown in (Table 1). Group A rats died at Day 8 of treatment while gradual increase in weight was observed in other Groups after treatment. The descriptive statistics and ANOVA of weight gain before and after treatment is shown in Appendix I-V which implies that there was significant difference at 0.05 level. The physical observation of the rat vital organs after treatment in all groups against the control which showed no difference is shown in (Table 2). Hematological parameters (full blood count) of treated rats are shown in (Table 3). The phytochemical constituents of the ethanol extract of *C. albidum* seed indicates the presence of flavonoids, tannins, phenols, steroids and saponins are shown in (Table 4). Biochemical tests results carried out on test bacteria is shown in (Table 5).

Antibacterial susceptibility analysis of ethanol extract

The antibacterial analysis of the ethanolic seed extract of *C. albidum* against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* is shown in (Table 6). The tested bacteria exhibited varying level of inhibition as indicated in (Table 6). The extract at all concentrations showed potent antibacterial activity against the tested bacteria. The Minimum inhibitory concentration of the ethanolic extract of *C. albidum* seed against the tested bacteria is shown in (Table 7). The extract showed better inhibitory against *Staphylococcus aureus* (31.3 mg/ml). The Minimum inhibitory concentration of the ethanolic extract against *Pseudomonas aeruginosa* and *Escherichia coli* was at 62.5 mg/ml. The Minum bactericidal concentration of extract against tested bacteria is shown in (Table 8). The MBC of the extract against *E. coli* was at 250 mg/ml, *S. aureus* was at 125 mg/ml and 500 mg/ml against *P. aeruginosa*.

DISCUSSION

The experimental animals (Wister rats) in all group (A, B and C) used were fed with different rations of *C. albidum seed.* Group A (20 g of *C. albidum* seed), Group B (10 g of *C. albidum seed* + 10 g of commercial animal feed), Group C (4 g of *C. albidum* seed + 16 g of commercial animal feed). The animals in group B and C fed on the seed mixed with a commercial animal feed while group A rats were observed to avoid eating the seed and only fed on it sparingly thereby leading to loss of weight, appeared depressed and eventual death at Day 8 of treatment. The rats were observed to have died not as a result of the seeds toxicity but due to starvation, which is an indication that the rats tends to feed on the seed when it is mixed with a commercial animal feed. Although, the control animals showed the highest weight gain, there was an observed significant weight gain in the experiment groups too. Analysis of blood parameter is relevant in risk evaluation as changes in the hematological system have higher predictive value for human toxicity when the data are translated from animal studies (Olson *et al.,* 2000). There was no significant difference in the hematological parameters of the treated group and the control group of rats. The non-significant effect of the seed on the RBC may be an indication that the balance between the rate of production and destruction of the blood corpuscles were not altered. *C. albidum* seed exerted a significant reduction in the platelets of rats in Group B which indicates thrombocytopenia. Platelet aggregation plays a pivotal role in the physiopathology of thrombotic diseases.

The histology (physical observation of the vital organs) of rats in the treated group showed no significance difference when compared with the control group, as the seed seem to have no effect on them. The phytochemical analysis revealed the presence of flavonoids, tannins, phenols, steroids and saponins. The flavonoid content of *C. albidum* could have been responsible for the antiplatelet effect in the group. Studies suggested various mechanisms by which flavonoid exert its antiplatelet property that is, by lowering intracellular Ca^{2+} levels; alteration in the metabolism of cAMP, and thromboxane A2 (Kang *et al.,* 2001). These results are similar to findings of Oputah *et al.* (2016) who reported the presence of saponins, carbohydrates, flavonoids, quinones, cardiac glycosides, fatty acids and terpenoids in ethanol extract of *C. albidum* seed. Adeleye *et al.* (2016) reported that the presence of flavonoids, steroids, alkaloids, tannin, anthraquinone and cardiac glycosides in fruit extract of *C. albidum*. Duyilemi and Lawal, (2009) also reported that the plant contains saponins, tannins and anthraquinone in the leaves of *C. albidum*. The ethanolic extracts of C. albidum seed were found to be effective at all concentrations against *P. aeruginosa*, *E. coli* and *S. aureus*. This is in agreement with findings ofAdeleye *et al.* (2016) who reported potent antibacterial effect of methanol and aqueous extract of *C. albidum* fruits against *Klebsiella pneumonia* and *S. aureus*. Oputah *et al.* (2016), in their study also revealed potent antibacterial efficacy of *C. albidum* ethanolic seed extract

Table 1. Effect of *Chrysophyllum albidum* on the weights of treated Wister.

Group	Before Treatment (g)	After Treatment (g)	Weight gain (g)
Group A	126.0 ± 4.00	-	-
Group B	128.5 ± 4.50	140.5 ± 4.50	12.0 ± 0.00
Group C	120.0 ± 9.00	128.5 ± 6.50	8.5 ± 2.50
Control	121.5 ± 3.50	139.0 ± 9.00	17.5 ± 5.50

Keys: Values are weight and weight gain ± Standard Error of Mean, n= 0.05
Group A- fed with 20 g of seed
Group B- fed with 10 g of seed + 10 g of commercial animal feed
Group C- fed with 4 g of seed + 16 g of commercial animal feed
Control- fed with 20 g of commercial animal feed

Table 2. Physical observation of Rats vital organs.

Group	Liver	Spleen	Kidney	Heart	Brain
Group B	Normal	Normal	Normal	Normal	Normal
Group C	Normal	Normal	Normal	Normal	Normal
Control	Normal	Normal	Normal	Normal	Normal

Table 3. Hematology of rats (full blood count).

GROUP	WBC $x10^9$/L	HGB g/dl	PCV %	MCV Fl	MCH Pg	MCHC g/dL	RBC $x10^6$/ul	Platelet count	NEU	LYM	MON
GROUP B	4.2	12.1	37.3	92.1	27.5	33.4	4.9	195	52	29	0
GROUP C	7.6	15.7	43	80.9	32.8	34.5	4.5	356	60	40	0
Control	6.2	15.5	40	85.9	27.1	32.9	4.9	340	58	38	0

KEY: WBC-White Blood Cell, HGB-Hemoglobin, PCV-Part Cell Volume, MCV-Mean Corpuscular Volume, MCHC-Mean Corpuscular Hemoglobin Concentration, RBC-Red Blood Cell, NEU-Neutrophil, LYM-Lymphocyte, MON-Monocyte.

Table 4. Phytochemical profile of *C. albidum* analysis.

Phytochemicals	Ethanol Extract
Alkaloids	-
Flavonoids	+
Terpenoids	-
Tannins	+
Phenols	+
Steroids	+
Saponins	+

Key: + Present; - Absent.

Table 5. Biochemical characteristics of tests bacteria.

Bacteria	Gram reaction	Citrate	Indole	Urease	Catalase
Escherichia coli	- rod	-	+	-	+
Staphylococcus aureus	+ cocci	-	-	-	+
Pseudomonas aeruginosa	- rod	+	-	-	+

Key: + = Positive; - = Negative

against *S. aureus*, *E. coli*, *P. aeruginosa*, *Proteus vulgaris* and *Micrococcus varians*. Okoli and Okere (2010) also reported that *C. albidum* root extracts and stem bark successfully inhibited *P. aeruginosa*, *E. coli*, *S. aureus*, C*lostridium tetani*, *Bacillus subtilis*, and *Candida albicans*, while the seed cotyledon inhibited *Candida albicans*.

Table 6. Antibacterial susceptibility analysis of ethanol extract of *C. albidum.*

Test Organisms	Concentration (mg/ml)	Ethanolic extract value
E. coli	500	17.0 ± 0.23
	250	14.0 ± 0.16
	125	12.0 ± 0.10
	62.5	10.0 ± 0.10
S. aureus	500	24.0 ± 0.00
	250	20.0 ± 0.12
	125	16.0 ± 0.49
	62.5	13.0 ± 0.05
P. aeruginosa	500	15.0 ± 0.05
	250	13.0 ± 0.11
	125	10.0 ± 0.06
	62.5	9.0 ± 0.44

Values are Zone Diameter of Inhibition ± Standard Error of Mean (SEM)
Key: mg/ml = milligram per milliliter

Table 7. Minimum inhibitory concentration of ethanol extract of *C. albidum.*

Test Organisms	Concentration (mg/ml)	Ethanol extract reaction
E. coli	500	-
	250	-
	125	-
	62.5	-
	31.3	+
	15.6	+
S. aureus	500	-
	250	-
	125	-
	62.5	-
	31.3	-
	15.6	+
P. aeruginosa	500	-
	250	-
	125	-
	62.5	-
	31.3	+
	15.6	+

Key: + Turbidity (Indicative of bacterial growth); - Less turbid (Indicative of inhibition)

Table 8. Minimum bactericidal concentration (MBC) of ethanol extract of *C. albidum*

Test Organisms	Concentration (mg/ml)	Ethanol extract reaction
E. coli	500	-
	250	-
	125	+
	62.5	+
	31.3	+
	15.6	+
S. aureus	500	-
	250	-
	125	-
	62.5	+
	31.3	+
	15.6	+
P. aeruginosa	500	-
	250	+
	125	+
	62.5	+
	31.3	+
	15.6	+

Key: + Growth; -No Growth (Indicating bactericidal activity)

Conclusion

The hematology result has demonstrated that the seed of *C. albidum* may not cause adverse effect on hematological indices of the rats. The histology of the rats' vital organs also showed no significance difference between the treated groups and control group, thus underlining its potential as an additive to animal feed. The extract showed potent inhibitory and bactericidal activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* exhibiting its potency as a possible source of antimicrobial targets. This could be attributed to the presence of the wide range of phytochemicals such as flavonoids, tannins, phenols, steroids and saponins.

Recommendations

The result of this study showed relatively safe effect on the hematological indices of the rats studied. It is recommended therefore,

I. That further studies be carried out to determine the toxicity of the *C. albidum* seed on rats.
II. Also, investigation on the nutritive and anti-nutritive values of the seeds should be determined.
III. The *in vivo* antimicrobial activity of the extract should be determined in further studies.

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