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Preliminary investigation into the allelopathic and cytotoxic activities of *Mucuna sloanei* and *Chrysophyllum africanum* seed coats methanol extracts in weed and pest control

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Abstract

The seed coat, as a primary defense structure for the seed against adverse environmental conditions, contains properties that contribute to seed quality and viability for germination, vigour and storage potential. They also contain fats (monounsaturated and polyunsaturated), vitamins, minerals and antioxidants. This work sought to investigate the allelopathy and cytotoxicity of the methanol extracts of *Mucuna Slonei* (MSCM) and *Chrysophyllum africanum* (CSCM) seed coats.

The seed coats of MSCM and CSCM were removed and macerated in 70 % methanol for 72 hrs to obtain crude extracts. Allelopathy activity was evaluated using germination rate and root elongation tests. While cytotoxicity test was carried out using the brine shrimp lethality assay.

Methanol extract of seed coats of MSCM and CSCM had no significant difference on maize root and shoot length when compared with the control. This implies that MSCM and CSCM seed coat exhibit no allelopathy activity. The MSCM and CSCM seed coat extracts however showed cytotoxicity activity at 0.6 mg/ml and 0.8 mg/ml respectively.

The seed coat could thus be useful in industries for the production of manure for promoting crop production. But the cytotoxic activity suggests its application in pest control and potential hazard to aquatic lives and the environment when improperly disposed.

Keywords: Allelopathy; Cytotoxicity; Seed coats; *Mucuna sloanei; Chrysophyllum africanum*; Extract; Brine shrimp

1 Introduction

Plants interact chemically, exerting stimulatory or inhibitory impact on surrounding vegetation. Cheng and Cheng (2015) described such plant interaction between donor and receptor plants which may exert positive effects (e.g., applied in weed control, agricultural management, or crop protection) or negative effects (e.g., biological invasion, autotoxicity, etc) as plant allelopathy [1]. That term first used by Molisch in 1937 is now a sub-discipline of chemical ecology [1]. Crop plants can naturally produce and exude allelochemicals into their surroundings, suppressing weeds in their vicinity. This explains the phenomenon of plant dominance, succession, formation of plant communities and vegetation climax, as well as enhanced crop productivity [2]. Allelopathy plays an important role in both natural and agro-ecosystems. Researchers can also manipulate allelopathy to improve crop productivity and environmental protection using eco-friendly control of weeds, pests, crop diseases, nitrogen conservation in crop land as well as the

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synthesise novel agrochemicals from natural products. Such areas of research have gained prominent attention in recent times [3]. Identifying and quantifying the bioactive allelochemicals in plant and environments are thus vital areas of studies in allelopathy so as to investigate, by an *in vitro* test, the effect of leaching toxic substances or their direct contact [1, 4].

Cytotoxicity is harmful activity on living cells by the action of chemotherapeutic agents [5]. When plant extracts or isolated biologically active compound are used as the test agent in initial step in cytotoxicity studies, invaluable insights in determining the potential toxicity of the extract is obtained. Given this information, the ability to accurately measure cytotoxicity can prove to be vital in identifying compounds that might pose health risks to humans [6]. Treating cells with a cytotoxic compound can result in a variety of fates for them. On contact with the cytotoxic compound, healthy living cells can either be induced to undergo necrosis (accidental cell death) or apoptosis (programmed cell death) [7]. Whereas apoptotic cell death is slower, orderly, and genetically controlled, cells undergoing necrosis, rapidly lose membrane integrity and die rapidly due to cytolysis. There is a decrease in the cell viability (stoppage in active growth and division [5].

Historically, the seed coats of *C. africanum* and *M. sloanei* have been seen as waste products disposed in the environment. This study however seeks to investigate the seed coats and its extract for cytotoxicity and allelopathy. The hazardous (allelopathic) effect will be evaluated using the dish pack. If hazardous, it could inhibit the growth of crop hence can be applied in herbicides. Cytotoxicity of extracts of the seed coats was determined using brine shrimp lethality bioassay, using *Artemia salina nauplii*. The result will evaluate the efficacy of the seed coat and seed extracts as possible sources of potential environment friendly herb and pest control agents.

2 Material and methods

All analyses were carried out in the Department of Biochemistry Laboratory, University of Uyo, Nigeria.

All reagents used were of analytical grade and purchased from a reputable supplier.

A measuring cylinder of 1000ml capacity was used to measure out 700ml of absolute methanol, then freshly prepared distilled water was added to make it up to 1000ml to prepare 70% methanol.

2.1 Collection of samples for analysis

The mature seeds of *Mucuna sloanei* and *Chrysophyllum africanum* used in this work were obtained from a local market (Itam market, GPS 5.04642, 7.89802) in Itu Local Government Area of Akwa Ibom state Nigeria on the 14th of December 2021. The *Mucuna sloanei* has a black seed coat when mature but is white to cream while young. The *Chrysophyllum africanum* is yellow when the fruit is matured and has 4 to 5 seeds inside and the seed has brown seed coat. The seeds were picked and cracked or de-shelled using a hammer after which the seed coats were separated from the seed. The seed coats were sun dried for about 20 minutes before continue drying at room temperature in the laboratory for the next 12 hrs.

2.2 Preparation of extract

Exactly 446 g of the pulverized *C. africanum* seed coat was weighed and macerated in labeled plastic bucket using 3.5L of 70 % methanol and covered then allowed to stand for 72 hrs with intermittent stirring. Similarly, 310 g of the pulverized *Mucuna sloanei* was weighed and macerated in 2.5 L of 70 % methanol in a well labeled plastic bucket with cover for 72 hrs. At the end of 72 hrs, each of the labelled micelle mixture (seed coat extract and methanol mix)was separated from the marc by decantation and evaporated in a water bath to remove the methanol content and obtain the crude extract.

2.3 Germination rate and root elongation tests

This test was performed in line with published method by Asya, *et al*. 2014 [8] Maize seeds were grown on cotton wool in a covered petri dish at room temperature. The test had a control (a petri dish with all components but no extract) while other petri dishes had the growing crop with extract at different concentrations (experimental doses). Four seeds of maize (Zea mays) were placed on cotton wool in each of the petri dishes (11 cm in diameter). Five ml of each concentration of extract or distilled water, as a control, was applied to the seeds. The dishes were sealed for 72 hr. The percent of germinated seeds was recorded, and the length of the roots and shoot of germinated seeds was measured. The percentage root growth inhibition in relation to the control for each extract was determined [8]. Seeds that did not germinate were excluded in the root elongation test. Three replications of each treatment were done. The planted seeds

were allowed to germinate for 4 days. The concentrations induced on the planted seed include 0.1, 0.2, 0.4, 0.6, and 1.0 mg/ml.

2.4 Preparation for cytotoxicity activity

The cytotoxic activity of *Mucuna sloanei* and *Chrysophyllum africanum* was evaluated using the brine shrimp lethality assay method as described by Michael [9]. The brine shrimp lethality bioassay is a simple, high throughput cytotoxicity test of bioactive chemicals. It is based on killing ability of the test compounds on a simple zoological organism-brine shrimp (Artemia salina). [6]. The brine shrimp lethality bioassay is widely used in the evaluation of toxicity of heavy metals, pesticides and medicines especially natural plant extract. Toxicity of each extract were tested at different concentrations of 0.2, 0.4, 0.6, and 0.8 mg/ml on the brine shrimps and three application were used for each concentration. The control was conducted in each case using distilled water. The results were subjected to a one-way ANOVA at a level of significance at 95% ($P \leq 0.05$)

3 Results

3.1 Allelopathy

The result of allelopathy activity of *Mucuna sloanei* (MSCM) and *Chrysophyllum africanum* (CSCM) seed coat methanol extract for the root is presented in figure 1. From this study, it was revealed that MSCM and CSCM seed coats have no allelopathic activity. In comparison with the control, methanol extracts of MSCM and CSCM seed coats showed no inhibitory or stimulatory effects on seed root length, and shoot length in the concentrations investigated. ($P \leq 0.05$).

Figure 1 is the graph containing the root length of MSCM and CSCM which shows that there is no significant difference between the different concentrations and the control, although the control is higher in height than the concentrations in the graph; this clearly shows that methanol extracts of MSCM and CSCM seed coats has no allelopathy activity on the root length of a germinating maize seed. Figure 1, also showed no significant difference between the different concentrations and the control, revealing clearly that methanol extracts of *Mucuna sloanei* and *Chrysophyllum africanum* seed coats has no allelopathy activity on the shoot height of a germinating maize seed as observed from the data. The different concentrations add little or no difference when compared with the control.

Therefore, the extracts at both low and high concentrations investigated have no allelopathy effect on the root and shoot maize seed germination and seedling growth.

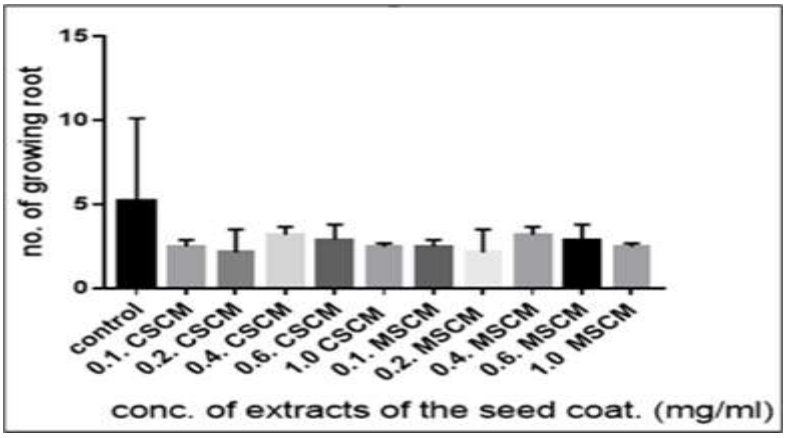

Figure 1 Bar graph showing allelopathic activity of the methanol extracts of *Mucuna sloanei* and *Chrysophyllum africanum* seed coats on root length of growing maize seeds

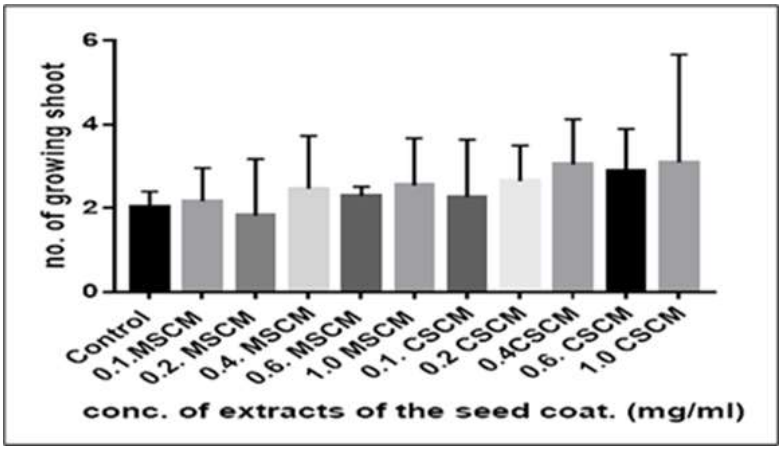

Figure 2 Bar graph showing allelopathic activity of the methanol extracts of *Mucuna sloanei* and *Chrysophyllum africanum* seed coats on shoot length of growing corn seeds

The result of cytotoxicity activity of *Mucuna sloanei* (MSCM) and *Chrysophyllum africanum* (CSCM) seed coat methanol extracts are presented in figure 3. From this study, it was revealed that MSCM and CSCM seed coats have cytotoxicity activity. This result shows that MSCM and CSCM seed coats have the highest significant cytotoxicity at 0.6 mg/ml and 0.8 mg/ml when compared to the control.

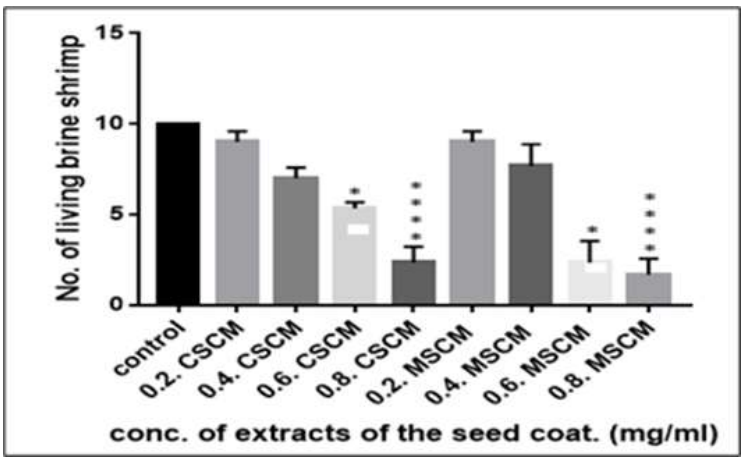

Figure 3 Bar graph showing cytotoxicity of methanol extracts of *Mucuna sloanei* and *Chrysophyllum africanum* seed coats on brine shrimp

4 Discussion

Allelopathy is one of the expressed phenomena of chemical interaction exhibiting widespread significance in natural eco-systems [10]. In nature, the released allelochemicals exert influence on other plants, weeds or micro-organisms in inhibitory or excitatory ways [10, 11]. The results presented here clearly showed that *Mucuna sloanei* and *Chrysophyllum africanum* seed coats extract have no differential allelopathic expressions. Ismail and Chong [12] in their work on allelopathy demonstrated that a reduction in speed of germination and radicle lengths of their test plants were due to allelochemicals in the extracts of seed coat, but not the osmotic potential.

Mucuna sloanei and *Chrysophyllum africanum* seed coats extract showed no allelopathic effects on root length and shoot height of the target specie, which agrees with previous studies [13]. Ismail and Mah 1993 [14], found similar results when testing the allelopathic effects of Mikania seed coat on root lengths of *Asystasia intrusa* BI., and *Paspalum conjugatum* Berg. In contrast, our result is not in agreement with the previous studies [10, 11]. However, Wang, 2004 [15] found that *M. micrantha* seed coat extract showed stronger allelopathic effects on root lengths. The absence of allelopathic effects of the seed coat extract might result from differences in concentrations of diffusible allelochemicals, varied chemical composition, or differentiated alkalinity [15] between seed coat extracts. Chemical isolation and

identification conducted simultaneously in seed coat extracts and similar field experiments are necessary to clarify our results. Certainly, more research needs to be done along the lines described in this section.

Cytotoxicity studies are a useful initial step in determining the potential toxicity of a test substance, including plant extracts or biologically active compounds isolated from plants [16]. The cytotoxicity test is an important *in vitro* biological evaluation system, just as other experimental methods to evaluate cytotoxicity are continuously being developed and improved with the progress of modern cell biology [16].

It is evident that different plant seed coats extracts were found to be lethal to brine shrimps indicating that the extracts are biologically active. The methanol extract was more active with higher concentrations whereas the lower concentrations were less active. The Brine shrimp lethality bioassay is a recent development in the bioassay for the bioactive compounds. The brine shrimp assay has advantages of being rapid (24 hrs), inexpensive and simple. It easily utilizes a huge number of organisms for statistical validation and requires no special equipment but relatively small amounts of sample. According to the degrees of activity of the extracts against the brine shrimps, it could be arranged in the order: methanol extract 0.8 > 0.6 > 0.4 > 0.2 mg/ml respectively. This result agrees with similar works *with* Ji-hoon, *et al* 2012 [17]. and Islam *et al*. 2000 [18] but in contrast with that of Sandeep and Vijay, 2019 [19] which may likely proceed from the process of extraction or due to the differences in solvent used for extraction (ethanol as against methanol) which may affect composition of the extracts.

This significant lethality of *Mucuna sloanei* and *Chrysophyllum africanum* methanol extracts to brine shrimp is indicative of the presence of potent cytotoxic components which warrants further investigation However, more comprehensive studies are needed in this line. Additionally, understanding the mechanisms involved in the cytotoxicity can likewise give researchers a more in-depth knowledge on the biological processes governing cell growth, cell proliferation, and death.

5 Conclusion

The seed coats of *Mucuna sloanei* and *Chrysophyllum africanum* like major seed coats of plants in Nigeria is under-utilized. It is believed that as more investigations is carried out on it, some of the myth surrounding it will be removed and their benefit will be harnessed profusely. It can be seen that C*chrysophyllum africanum* and *Mucuna sloanei* seed coat extract have no allelopathy activity but have strong cytotoxicity activity. Therefore, these plant parts will not find usefulness when trying to improve or inhibit the growth of crops. On the other hand, *Chrysophyllum africanum* and *Mucuna sloanei* seed coat extracts were found to have strong cytotoxic activity at a higher concentration on the brine shrimp.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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