EFFECTS OF CUSTARD APPLE (*Annona squamosa L.*) SEED EXTRACTS ON DANDRUFF (*Malassezia globosa)*

Alıyu Nafısatu* and Dunıya Anthony Vıncent

*Corresponding author's Email: nafnaf4u@gmail.com

Abstract: Scalp is unique and characterized by thick layer with high follicules and sebaceous glands which makes it more prone to mycotic infections like dandruff. Dandruff is a fungal infection characterized by itchy, flaking skin without visible inflammation. Currently availabe treatments have limitations either due to poor clinical efficacy or due to the compliance issues. Furthermore these drugs are unable to prevent recurrence. However this research was aimed to ascertain the potential of custard apple (*Annona squamosa L)* seed phytoconstituents extracts on dandruff causing *Malassezia globosa*. Standard extraction methods were employed to obtain each extract after maceration. *M. globosa* was cultivated from scrapings of dandruff and sensitivity test was carried out.The Ethyl acetate, Ethanol-distill water, Acetone and Methanol extracts were not effective on *Malassezia globosa,* however, Ethanol extract was more effective than the crude extract. Ethanol extract had inhibition zones of 35, 20, 14, 12 mm at 100, 50, 25, 12.5 mg/ml respectively while crude extract had zones of 15, 12, 10 and 0 mm at 100, 50, 25 and 12.5 mg/ml respectively. Hence Ethanol extract of custard apple seed is recommended for treatment of dandruff infection. Further research is however needed to establish the bioactive principle of this study.

Keywords: *Annona squamosa L.*, Dandruff, Extraction, Phytochemicals, Sensitivity test

1. INTRODUCTION

Scalp disorders are physical illness not severe to cause morbidity, but of great social concern. Scalp disorders include fungal and bacterial colonies infestations that cause abnormalities like alopecia, seborrheic dermatitis, ring worm, scalp psoriasis, scalp folliculitis, head lice and dandruff (Narshana and Ravikumar, 2019). Custard apple belongs to the family Annonaceae botanically known as *Annona squamosa L*. It has been known as sweetsop and sugar apple, it is a green, cone-shaped fruit with scaly skin and creamy sweet flesh (Yathish *et al*., 2013). Custard apple seeds have been used as folk medicine worldwide. In traditional medicine, custard apple seeds were mainly used to treat various digestive disorders. It was observed from past reports that the extracts of seeds of custard apple have good antibacterial properties (Ristiati *et al*., 2018) and based on research (Febrianni, 2011) the aqueous extracts and organic extract solvent from parts of the plant of custard apple reported to be an insecticide, inhibiting the activity of insect feeding and repellent to some kind of important pest of agriculture and pest in storage, hence the need to ascertain the effectivity of some bioactive fractions on dandruff causing microorganism.

Phytochemicals are important secondary metabolite found in medicinal plants that are non essential for the normal functioning of the human body. They are bioactive nonnutrient component of plants that may have beneficial (or harmful) health effects, example are alkaloid, flavonoid, saponin, terpenoid, cardiac glycoside, and so on. These can be obtain by suitable extraction methods using solvent depending on the polarity of the component of interest. Sensitivity testing is a measurement of the susceptibility of bacteria/fungi to antibiotics. This is used to determine which extract will

inhibit the growth of fungi causing a specific infection.

2. METHOD

Sample collection and Preparation

Custard apple (*Annona squamosa L*) fruits were harvested from the local garden in Kabama layout, Sabon gari, Zaria. The fruits, in full maturity, were washed under running water then sliced and the seeds were separated with a steel spoon. The seeds were collected and cleaned from the residue of fruit, then washed completely with tap water and air dried. The dried seeds were blended, sieved and stored in tight jar until used.

Custard apple seeds were crushed to powder using electronic blender, sieved with 20mm mesh size sieve to obtain the powder form. Maceration was carried out, where ten grams (10g) powder sample are weighed into a stoppered container with mixture of solvent and allowed to stand for a period of 7 days with frequent agitation, until soluble matter dissolved. The mixture was then filtered with filter paper and further use to obtain fraction of each component.

2.1 Sample Extraction

2.1.1 Ethyl acetate Extract

Ten grams of custard apple seed powder was weighed into stopped sample bottle, 80% ethyl acetate, ethanol, acetone and water was added, allowed to stand for 7 days, heated at 40 ^{o}C for 30mins, allowed to cool and filtered. The extract was dissolved in 20ml water, partitioned by mixing 20ml diethyl ether in separating funnel to remove pigments and lipids. The aqueous layer was placed in another separating funnel and made alkaline by adding dilute ammonia solution. Chloroform (50ml) was added, shook gently and allowed to separate. The lower chloroform layer containing Ethyl acetate was run off into a beaker and concentrated to dryness (Trease and Evans, 1989).

2.1.2 Methanol Extract

Ten grams of custard apple seed powder was weighed into stopped sample bottle, 30% ethanol, methanol, acetone and water were added, allowed to stand for 7 days, heated at 40 ^{o}C for 30mins, allowed to cool and filtered. The extract was solubilized in 50ml of water/ethanol (95:5v/v) and extracted three times with chloroform (50ml) to remove lipophilic material. Then the aqueous phase was extracted three times with ethyl acetate (50ml) to obtain two distinctive fractions. The organic fraction was concentrated to dryness and used (Wen *et al*., 2020).

2.1.3 Acetone Extract

Ten grams of custard apple seed powder was weighed into stopped sample bottle, 30% ethanol, methanol, acetone and water were added, allowed to stand for 7 days, heated at 40 oC for 30mins, allowed to cool and filtered. Extract was defatted with 20ml petroleum ether. Extract was dissolved in water and shaken with n-butanol. N-butanol aliquots are combined and the liquids removed to give Acetone extract. Diethyl ether was used to precipitate (Usman *et al.*, 2018).

2.1.4 Ethanol Extract

Ten grams of custard apple seed powder was weighed into stopped sample bottle, 70% ethanol, water, ethyl acetate and acetone were added, allowed to stand for 7 days, heated at 40 ^{o}C for 30mins, allowed to cool and filtered. To 25ml extract, 2.5ml solution of 10% sulphuric acid was added, stirred and kept in hot water bath for 5min after which it was filtered. The filtrate was allowed to cool,

1ml 10% ammonia was added and shaken. Filtrate changed to pinkish red, indicative of glycoside (Sakalpanich and Gritsanapan, 2008) which was concentrated by heating on water bath.

2.1.5 Ethanol-distill waters Extract

Ten grams of custard apple seed powder was weighed into stopped sample bottle, 70% ethanol, water, ethyl acetate and acetone were added, allowed to stand for 7 days, heated at 40 ^{o}C for 30mins and allowed to cool then filtered. Distilled water (400ml) was added to 25g extract, partitioned with 150ml of diethyl ether three times. The aqueous fraction was partitioned with ethyl ether 150ml × 3. The aqueous fraction was further partitioned with N-butanol (150ml × 3) saturated with water. The butanol fraction was partitioned with 1% potassium hydroxide 50ml × 3. The potassium hydroxide fraction was acidified with dilute hydrochloric acid. The N-butanol fraction contain flavonoids (Usman *et al.,* 2018).

2.1.6 Crude

Ten grams of custard apple seed powder was weighed into stoppered sample bottle, 70% ethanol, water, ethyl acetate and acetone were added, allowed to stand for 7 days, heated at 40 oC for 30mins and allowed to cool then filtered.

2.2 Cultivation of *Malassezia globosa*

Scalp scrapings of dandruff was grown on potato dextrose agar and incubated for 3 days at room temperature (Murarkar *et al.,* 2018).

2.2.1 Direct Microscopy of Fungi

Fungal culture was taken on slide containing a drop of 10% potassium hydroxide with methylene blue and covered with cover slip. The slide was heated over the flame to remove air bubbles and observed under 10X and 45X objective lenses of microscope. Direct microscopy shows the typical mixture of organisms present. The presence of globose blastoconidia and pseudo- mycelia coupled with mycelia in the form of 'spaghetti' indicate the most identifiable difference of *M. globosa* from the other species of Malassezia (Murarkar *et al.,* 2018).

2.3 Antifungal Activity

Antifungal activity of custard apple seed extracts was tested against dandruff causing microorganism (*Malassezia globosa*) using Muller Hington agar (MHA) by well diffusion method. Fungal lawn of 0.1 ml each of fungal culture was made on separate solidified MHA plates. A well was made in the centre with the help of sterilized borer, 0.5 ml each of different concentrations (12.5, 25, 50 and 100mg/ml) of the extract was poured in the wells of separate MHA plates. A positive control with ciprofloxacin drug was prepared. All the fungal culture plates were incubated at 37 ^{o}C for 2-4 days. After incubation the plates were observed for zone of inhibition (Murarkar *et al.,* 2018).

3. RESULTS AND DISCUSSION

The result of morphology and view under light microscopy show hyphae and some spores which correspond to sphagetti and meatball appearance of mycelia and microconidia of *M. globosa,* this is similar to report by Isa *et al*., (2013) who isolated and identified *Malassezia globosa* associated with dandruff.

Inhibitory studies showed that Ethyl acetate, Ethanol-distill water, Methanol and Acetone extracts were not effective on *M. globosa* hence the 0mm zone of inhibition. However, Ethanol extract was effective on *M. globosa* with zones of inhibition 35, 20, 14, 12mm at 100, 50, 25, 12.5mg/ml respectively. This

may be due to the presence of component that are steroids having the ability to exert specific powerful action by disrupting structure and function of cell wall/membrane, mitochondria, biofilm, and mycotoxin synthesis, hence a very small amount can exert beneficial simulation. This study is different from Murarkar et al.,(2018), who reported 0.5ml each of lemon juice, neem leaves, onion juice, yoghurt and marketed shampoo with inhibition zones of 18, 13, 20, 18 and 15mm against *M. globosa*. The crude extract also show inhibition zones of 15, 12, 10 and 0mm at 100, 50, 25 and 12.5mg/ml respectively. Decreased activity when compared with Ethanol extract may be due to combined effect/interference from other inactive phytochemical components as shown in Table 1. The control (Ciprofloxacin) had inhibition zone of 35mm, whose effect can only be compared with 100mg/ml of Ethanol extract.

Table 1: Inhibitory activity of *A. squamosa* L seed extracts on *M. globosa*

Extracts	**Zone of inhibition (mm) at varying concentration of the extracts (mg/ml)**				
	100	50	25	12.5	Control (Ciprofloxacin)
Ethyl acetate	0	0	0	0	35
Ethanol-distill water	0	0	0	0	35
Acetone	0	0	0	0	35
Methanol	0	0	0	0	35
Ethanol	35	20	14	12	35
Crude	15	12	10	0	35

Table 2 show the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extracts against *M. globosa.* Ethyl acetate, Ethanol-distill water, Acetone and Methanol extracts were not effective, that is no activity, the extracts in the tubes became turbid indicating growth of *M. globosa* for both MIC and MFC. The MIC for Ethanol and crude extracts are 25 and 50mg/ml while the MFC are 50 and 100mg/ml respectively that is, the test tubes were clear (growth inhibition), these concentrations signify activity. This study is in accordance with Afzal *et al*., (2022) who reported antifungal properties of glycosides isolated from *Albizia kalkora*. This study is in contrast with Vu *et al.*, (2021) who reported MIC of 2.5µl/ml of *M. arvensis* essential oil on *Malessezia* genus fungi.

Table 2: Inhibitory concentration of *A. squamosa* seed extracts on *M. globosa*

Extracts	**Concentration of extract (mg/ml) for:**	
	MIC	MFC
Ethyl acetate	-	-
Ethanol-distill water	-	-
Acetone	-	-
Methanol	-	-
Ethanol	25	50
Crude	50	100
KEY: - means no activity		

4. CONCLUSION AND RECOMMENDATIONS

From this research it is conclusive that Ethyl acetate, Ethanol-distill water, Acetone and Methanol extracts were not effective on *Malassezia globosa*, however, demonstrated that Ethanol extract contain potent phytochemical that can inhibit the growth of *Malassezia globosa*. Therefore, this study recommend the use of custard apple seed extract in cosmoceuticals such as shampoos, creams and so on to treat *Malassezia* fungal infections. Sensitivity test should however be carried out on other *Malassezia* strain so as to ascertain the extracts' effect. Further research work should also be carried out to determine the active glycoside responsible for this activity.

AUTHOR CONTRIBUTION

Author 1: conceptualization, methodology, analysis, investigation, writing – original draft preparation, writing – review and editing. Author 2: methodology and sourcing reagents.

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