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Research Article

Physicochemical, Antimicrobial and Toxicity Assessments of *Anacardium occidentale* Cream Formulations

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Abstract

Anacardium occidentale is used locally for treating toothaches, gum problems, cough and bronchitis, diarrhea, dysentery, haemorrhoids, rheumatism, arthritis, eczema, psoriasis and skin infections. There are increasing global investigations on incorporation of medicinal plants into conventional pharmaceutical dosage forms for health and safety reason. Therefore, this study aimed at formulating and assessing herbal creams of *A. occidentale* leaf extract. Creams were formulated from the ethyl acetate fraction of *A. occidentale* leaf using standard procedures. Three creams containing the leaf extract in 2.5, 5 and 10% w/w were prepared using aqueous cream BPC as base. Organoleptic properties, density, extrusion time, spreading length, pH, diffusion rate, viscosity and globule size were assessed for the creams. Their antimicrobial effect was tested using *Staphylococcus aureus* ATCC 2785, *Pseudomonas aeruginosa* ATCC 29213, *Trichophyton rubrum*, *Epidermophyton* sp. and *Candida albicans*. The formulated creams were smooth, dark yellow to deep brown, with density ranging from 0.78±0.10 to 0.98±0.01 g/cm3, extruding time of 4.81±0.27 to 6.01±0.23 sec, spreading length of 3.93±1.03 to 4.28±0.61 cm, pH of 3.58±0.02 to 4.01±0.03, diffusion rate of 2.11 to 3.00 mm/hr, viscosity of 622.50±3.54 to 1277.50±307.59 cP and globule size of 55.38±42.65 to 188.4±98.88 µm. Their zones of inhibitions were 13.3-16.7 mm for bacteria and 9.7-16.0 mm for fungi. There was neither oedema nor erythema on the mice skins. The formulated herbal creams have acceptable physicochemical properties, active against tested bacteria and fungi and demonstrated safety profiles in animal model. The creams could be further enhanced for translational outcomes.

Key Words: *Anacardium occidentale*, Herbal creams, Physicochemical properties, Antimicrobial activity, Toxicity

INTRODUCTION

Dermatophytosis is a transmissible disease affecting the nails, hair and skin; and it is among the mainly widespread, superficial and cutaneous fungal infection in man (Sultan *et al*., 2020). Superficial mycosis is common worldwide and their epidemiological characteristics are different in different geographical areas (Araya *et al*., 2021). The World Health Organization estimated about twenty to twenty-five per cent worldwide incidence of dermatomycosis (Brasch and Glaser, 2019). Dermatophytes can affect all age groups and genders (Araya *et al*., 2021). However, new drugs are often not affordable in developing countries particularly in West Africa, consequently up to eighty per cent of the populace use medicinal plants as therapies (Li *et al*., 2020).

Herbal remedies for skin care with antimicrobial activities have been prepared from different plant parts and topically administered as solvent extracts, creams, ointments, lotions, gels or soaps (Ajala *et al*., 2016). Creams and gels have higher viscosity unlike lotions which have low viscosity and are applied to unbroken skin (McDonald, 2016). Creams are non-greasy with opaque appearance while ointments are more viscous and translucent. They also contain more than forty per cent water and volatiles while ointments have less than twenty per cent water and volatiles (Barnes *et al*., 2021). Ointments are suited for chronic, dry lesions and contraindicated in exudative lesions while creams are suitable for oozing wounds (Ajala *et al*., 2016). Some of the factors responsible for penetration of topical formulations into the skin are based on the physicochemical properties of drug substances such as: drug solubility, concentration, particle size, partition coefficient, pH, polymorphism and molecular weight (Supe and Takudage, 2021).

Over the years till date, herbal cream formulations have been attempted by many researchers for improved healthcare. For examples, an anti-wrinkle cream was formulated by Soisuwan *et al*. (2010) from *Caesalpinia pulcherrima*, Kale *et al*. (2011) created sunscreen cream from *Tagetes erecta*, Nair *et al*. (2012) produced skin protection cream from *Curcuma longa*, Manimaran *et al*. (2014) formed wound healing creams from *Eupatorium glandulosum* and *Cissus quadrangularis*, Majekodunmi and Nubani (2014) formulated anti-skin infection creams from *Acalypha wilkesiana*, while an anti-acne cream from *Thymus vulgaris*, *Rosa centifolia*, *Rosmarinus officinalis* and *Saponaria officinalis* was formulated by Mirela *et al*. (2016). Satpute and Kalyankar

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(2019) formulated anti- acne cream from *Boswellia serrata oil*. Shivathaya *et al*. (2022) formulated polyherbal face cream using ethanol extracts of Aloe vera gel, neem, turmeric and mint while Davkhar *et al*. (2023) formulated multipurpose herbal cream from turmeric, papaya, aloe-vera, neem and tulsi. Methanol leaf extracts of *Tephrosia vogelii* for topical application was formulated by Mlozi *et al*. (2023).

Anacardium occidentale Linn., a member of Anacardiaceae family, is a spreading, low-branched, evergreen (perennial), medium-sized tree that is majorly cultivated for its nut (cashew nut) and pseudo fruit (cashew apple). It originated from Brazil and has spread throughout Asia and Africa. Various parts of the plant have been engaged in different parts of the world for treating ailments and diseases such as cold and sore throat, malaria and typhoid, cough and bronchitis, diarrhoea and dysentery, rheumatism and arthritis, eczema and ringworms, corns, leprosy and skin challenges (Orwa *et al*., 2009; Aderiye *et al*., 2015; Erhenhi *et al*., 2016). Apple extracts of *A. occidentale* contain cosmetic and antimicrobial properties (Goncalves and Gobbo, 2012). Moreover, the leaf extracts of *A. occidentale* have phytochemical compounds such as carotenoids, tannins, flavonoids, saponins, alkaloids, steroids, anthraquinones and terpenoids, and also showed antibacterial activity (Aponjolosun and Fasola, 2020). Nowadays, more people prefer formulated herbal creams to synthetic or purely chemical creams for health and safety reason (Ekor, 2014; Satpute and Kalyankar, 2019); this implies the need for formulating the plant into acceptable dosage type. Thus, this investigation formulated *A. occidentale* leaf into creams, evaluated their physicochemical properties, antimicrobial ability and toxicity on mice skin.

MATERIALS AND METHODS

Materials: The plant material was the leaves of *Anacardium occidentale* obtained within the University of Ibadan; chlorocresol, emulsifying wax, liquid paraffin, propylene glycol, methanol and ethyl acetate were obtained from BDH Chemicals (England). Iron III chloride hexahydrate and hydrochloric acid were also procured from BDH Chemicals (England). In addition, *S. aureus* ATCC 2785, *P. aeruginosa* ATCC 29213, *Trichophyton rubrum*, *Epidermophyton* sp. and *Candida albicans* were obtained from the Department of Medical Microbiology, College of Medicine, University College Hospital (UCH), Ibadan. Furthermore, male Swiss mice were obtained from the Animal House of College of Medicine, University of Ibadan. Distilled water was obtained from the Department of Pharmaceutics & Industrial Pharmacy, University of Ibadan.

Preparation of plant samples: The leaves of *Anacardium occidentale* were authenticated and deposited in the University of Ibadan Herbarium (UIH 22489), Ibadan, Nigeria. The method of Aponjolosun and Fasola (2020) was adopted for plant preparation and extraction and hereby described. The leaves were washed with distilled water, air-dried under a shade for two weeks, ground with a milling machine (Disk Mill Pulverisette; FRITSCH GmbH, Deutschland) at the Department of Chemistry of the same institution. One kilogram (1 kg) from the milled leaves was soaked with three litres of methanol (BDH, England; CAS number 67-56-1) for forty-eight hours, filtered with Whatman filter paper No. 1 and the filtrate was concentrated with a rotary evaporator at 40 ^{o}C and 190.8 mmHg. The methanol extract was successively fractionated with n-hexane and ethyl acetate; at the Department of Chemistry of the same institution, concentrated and used for the formulation.

Formulation of creams: The base for the creams was prepared following standard procedures by British Pharmacopoeal (1979). Briefly, chlorocresol (0.1 %w/w) was dissolved in warm distilled water (69.9 %w/w), mixed with hot melted emulsifying ointment (30 %w/w) and thereafter constantly stirred till cold. Propylene glycol (10 %w/w) was mixed with 2.5, 5 and 10 %w/w ethyl acetate leaf fraction of *A. occidentale* and respectively incorporated into 87.5, 85 and 80 %w/w aqueous base BPC by constant trituration until pleasing products were formed (Ajala, 2014; Ajala, 2016). The creams were respectively labelled FAo1, FAo2 and FAo3 based on the concentration of the *A. occidentale* in each formulation. They were distributed into plain tubes for diverse analyses such as density, extruding time, spreading length, pH, diffusion rate, globule size, viscosity, antimicrobial and dermal toxicity tests.

Density, extruding time and spreading length: A 2 mL syringe was weighed, filled with 2 mL of the cream and re-weighed. The density was recorded and mean calculated. Density (g/cm3) = (W2 – W1) / V. Where W1 is the weight of the syringe only, W2 is the weight of the syringe and cream, V is the volume of the cream. The time taken to empty the cream from the syringe was also recorded and the mean calculated represent its extruding time. The cream (0.3 g) was put at the centre of a glass slide (20 x 5 cm), covered with another glass slide and a known weight (142 g) was placed on it for 5 minutes for uniform spread. The lengths of the spread cream were measured and the mean was calculated.

The pH and diffusion rate: The cream pH was determined by a Jenway pH meter (Model 3520, Essex, UK) at 25 ± 2 ^{o}C; after calibration, its electrode was dipped into the cream sample and the mean from five measurements was calculated. Melted nutrient agar (20 mL) was poured and allowed to set in a glass Petri dish. It was filled with 5 % w/v Iron III chloride hexahydrate in excess; the surplus solvent was drained off the dish and air dried. Three holes were bored into the agar with a cup borer (6 mm), filled with the *A. occidentale* cream and incubated at 37 ^{o}C. The diameters of the diffused cream were measured at varying times of one to forty hours, the calculated means were plotted on a graph, and its slope was determined (Femi-Oyewo *et al*., 2013).

Globule size and Viscosity: Each formulation of *A. occidentale* cream was stained with gentian violet and thinly smeared on glass slides. Pictures were taken with a digital microscope (VJ-2005 DN model Bio-microscope®) and the globule diameters of one hundred particles were randomly determined by TS View CX Image® Software, version 6.2.4.3 and Motic Image 2000 (China). Determination of the *A. occidentale* cream viscosity was done with a viscometer at 28 ± 2 ^{o}C using spindle number 7 at 50 rotations per minute (rpm). The attached spindle on the viscometer was dipped vertically into the cream without touching the bottom of the tube and readings were recorded. However, the preliminary viscosity

studies of the cream at 2.5, 4, 5, 10, 20, 50 and 100 rpm were done and the graph of viscosity against rotational speed was plotted (Ajala *et al*., 2016).

***In vitro* antimicrobial test of the leaf extract and the creams:** *Staphylococcus aureus*, *S. aureus* ATCC 2785, *Pseudomonas aeruginosa*, *P. aeruginosa* ATCC 29213, *Trichophyton rubrum*, *Epidermophyton* sp. and *Candida albicans* were obtained from Department of Medical Microbiology, College of Medicine, University College Hospital (UCH), Ibadan. Concentrations of 200, 100, 50, 25, and 12.5 mg/mL were prepared from the ethyl acetate fraction of A. occidentale. Agar well diffusion technique with a 6 mm cork-borer was used. Also, each of the formulated *A. occidentale* cream (0.5 g) was filled into a well, diffused for 30 minutes and incubated for 48 hours at 25 ^{o}C and 24 hours at 37 ^{o}C for fungi and bacteria, respectively. Amoxillin tablet and ketoconazole tablet were used as the control for the plant extract experiments while mycozoral cream and tydineal cream were the control for the formulated creams experiments. The zones of inhibition were measured in mm and recorded.

***In vivo* antimicrobial test of the creams:** Ethical approval (UI-ACUREC/App/2016/033) for animal experiment was obtained from the Animal Care and Use Research Ethics Committee, University of Ibadan. The Guide for the Care and Use of Laboratory Animals were followed (Garber *et al*., 2011). The mice were acclimatized for fourteen days, giving food and water *ad libitum* during the experiment. Twenty-five male Swiss mice (15 - 20 g) were procured and grouped into five animals per cage. *Staphylococcus aureus* (a log-phase culture of dilution 10-8 CFU, 0.5 mL) was intra-dermally injected into the clean-shaven marked out lateral part of the mice. The animals were treated after 24 hours of inoculation. Once in a day, 200 mg of FAo1, FAo2, FAo3 or tydineal cream was applied topically on the mice for three days while the untreated group served as the control. On the fourth day, the animals were sacrificed by quick cervical dislocation and decapitation. Their treated skin areas were excised and put inside sterile peptone water (2 mL). Serial dilution (0.1 mL from the peptone water added to 9.9 mL sterile peptone water) was done, 0.2 mL from it was cultured on Mannitol Salt Agar and incubated at 40 - 45 ^{o}C for 24 hours. A colony counter was used for the viable count of S. aureus and the percentage of the surviving bacteria was calculated (Gisby and Bryant, 2002; Ajala *et al*., 2016).

Dermal toxicity test of the creams: Twenty male mice (7 – 12 g) were procured, grouped into four (n = 5), they were given food and water ad libitum during acclimatization (fourteen days) and experimentation. The untreated group was the control. The side (20 mm diameters) of each mouse was clipped free of fur and 200 mg cream was applied on the skin. The creams were continually applied once in a day to the same sites on the mice for three weeks. The behavior of the mice was observed and the skin sites of application were visually assessed for any redness or oedema. The mice were decapitated and the sites of the harvested skins were kept inside 10 % formalin, histologically prepared and microscopically examined (OCED, 2002; Kamkaen *et al*., 2007; Ajala *et al*., 2016).

Statistical analyses: The mean values of viscosity studies at different rotational speed, physicochemical and the antimicrobial readings were analyzed with Microsoft Office Excel Software while Prism Software was used to calculate the mean values of globule size. The data were tested ($p < 0.05$) with unpaired student's t test, analysis of variance and Turkey Kramer's multiple comparison tests.

RESULTS

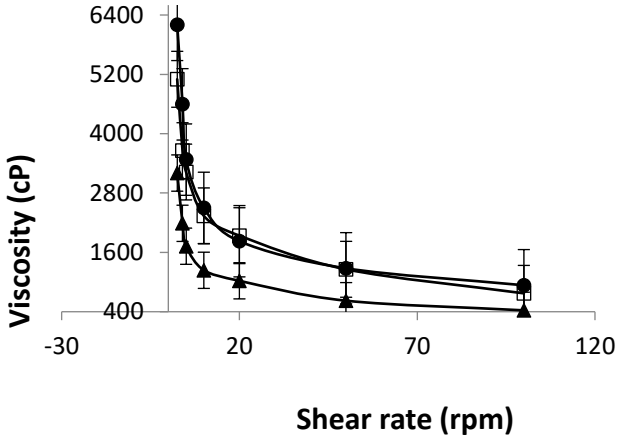
The physicochemical properties of the oil-in-water creams are shown in Table 1. All batches of creams formulated were water-washable and gave pleasant outlook with characteristic odour and smooth texture. The FAo1 cream had the highest density, extruding time, spreading length, diffusion rate and viscosity while FAo2 cream was highest in globule size.

Table 1:
Physicochemical properties of *Anacardium occidentale* cream formulations

Parameters	FAo1	FAo2	FAo3	EA
Colour	Dark yellow	Light brown	Deep brown	Deep brown
Density (g/cm^3)	0.98 ± 0.01	0.93 ± 0.03	0.78 ± 0.10	NA
Extruding time (sec.)	6.01 ± 0.23	5.81 ± 2.78	4.81 ± 0.27	NA
Spreading length (cm)	4.28 ± 0.61	3.93 ± 1.16	3.93 ± 1.03	NA
pH	3.58 ± 0.02	4.01 ± 0.03	3.77 ± 0.03	4.92 ± 0.14
Diffusion rate (mm/hr)	3.00	2.89	2.11	NA
Globule size (μm)$^+$	74.41 ± 72.24	188.40 ± 98.88	55.38 ± 42.65	NA
Viscosity (cP) at 50 rpm	1277.50 ± 307.59	1257.50 ± 251.02	622.50 ± 3.54	NA

Mean ± SE, n = 4, '+' means n = 100; FAo1, FAo2, FAo3 contained 2.5, 5, 10 % ethyl acetate leaf fraction of *A. occidentale* (EA) respectively; NA = not applicable

The rheological profiles of *A. occidentale* cream formulations are shown in Figure 1. The graph of viscosity against rotational speed of FAo1 cream was higher than FAo2 cream, except at 20 rpm while FAo3 cream was exceptionally the lowest at every point.



● FAo1 □ FAo2 ▲ FAo3
FAo1, FAo2, FAo3 have 2.5, 5, 10 % ethyl acetate leaf fraction of *A. occidentale* respectively.

Figure 1: Rheological profiles of *Anacardium occidentale* cream formulations (FAo)

The antimicrobial properties for the leaf ethyl acetate fraction of *A. occidentale* are shown in Table 2. The leaf had the highest zone of inhibition against *C. albicans* (24 mm) at 200 mg/mL. This was followed by 18 mm against *T. rubrum* while the same zone of inhibition (14 mm) was recorded against *P. aeruginosa* and *P. aeruginosa* ATCC 29213 at 200 mg/mL. Likewise, the same zone of inhibition (10 mm) was recorded against *S. aureus* and *S. aureus* ATCC 27853 while the least zone of inhibition (8 mm) was seen against *Epidermophyton sp*ecies at 200 mg/mL. Generally, the *A. occidentale* leaf fraction was more active on all the bacteria at 200 mg/mL, except on *S. aureus*, than the amoxillin tablet (control). Likewise, it performed better on *T. rubrum* and *C. albicans* at 200 mg/mL than ketoconazole tablet (control).

Table 2:
Antimicrobial activity of *Anacardium occidentale* leaf ethyl acetate fraction

Microorganism	***A. occidentale* ethyl acetate fraction**					**AmT**	**KeT**
	Concentration (mg/mL)						
	12.5	**25**	**50**	**100**	**200**	**0.020**	**50**
	Zones of inhibition (mm)						
Staphylococcus aureus	4	6	6	8	10	8	NA
Staphylococcus aureus (ATCC 2785)	4	6	8	8	10	10	NA
Pseudomonas aeruginosa	6	8	10	12	14	8	NA
Pseudomonas aeruginosa (ATCC 29213)	6	8	10	12	14	10	NA
Tricophyton rubrum	6	8	10	14	18	NA	10
Epidermophyton sp.	-	-	4	6	8	NA	10
Candida albicans	4	8	18	20	24	NA	10

ATCC = American Type Culture Collection, AmT = Amoxillin tablet, KeT = Ketoconazole tablet, NA = Not applicable, - = No inhibition.

Table 3:
Antimicrobial properties of *Anacardium occidentale* creams

Microorganisms	**FAo1**	**FAo2**	**FAo3**	**Tydineal**	**Mycozoral**
	Zones of inhibition (mm)				
Staphylococcus aureus	14.0 ± 0.0	15.3 ± 1.2	16.7 ± 1.2	33.7 ± 0.6	NA
Staphylococcus aureus[a]	15.3 ± 1.2	16.3 ± 2.1	14.0 ± 0.0	35.7 ± 0.6	NA
Pseudomonas aeruginosa	13.3 ± 1.2	14.0 ± 0.0	14.0 ± 0.0	37.7 ± 0.6	NA
Pseudomonas aeruginosa[b]	14.3 ± 2.1	15.0 ± 1.0	13.3 ± 3.1	37.7 ± 0.6	NA
Tricophyton rubrum	10.0 ± 0.0	12.0 ± 0.0	10.0 ± 0.0	NA	25.7 ± 0.6
Epidermophyton sp.	9.7 ± 0.6	10.0 ± 0.0	-	NA	27.7 ± 0.6
Candida albicans	11.7 ± 0.6	14.0 ± 0.0	16.0 ± 0.0	NA	27.7 ± 0.6

Mean ± SE, n = 3; FAo1, FAo2, FAo3 contained 2.5, 5, 10 % ethyl acetate leaf fraction of *A. occidentale* respectively. a = ATCC 2785, b = ATCC 29213. ATCC = American Type Culture Collection. NA = Not applicable.

The antimicrobial properties of *A. occidentale* formulated creams are shown in Table 3. The FAo3 cream had the highest zone of inhibition on *S. aureus* (16.7 ± 1.2 mm), whereas FAo1 cream produced the least inhibition (14.0 ± 0.0 mm). The FAo2 cream had the highest zone of inhibition on S. aureus ATCC 27853 and *P. aeruginosa* ATCC 29213 (16.3 ± 2.1 and 15.0 ± 1.0 mm, respectively) but FAo3 cream gave the least inhibition on the two microbes (14.0 ± 0.0 and 13.3 ± 3.1 mm, respectively). The FAo2 and FAo3 creams gave the same zone of inhibition against *P. aeruginosa* (14.0 ± 0.0 mm).

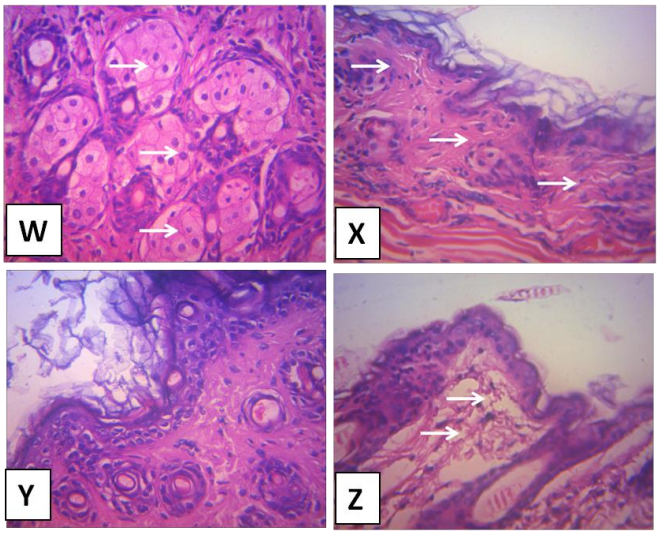
Moreover, FAo2 cream had the highest zone of inhibition on *T. rubrum* and *Epidermophyton* species (12.0 ± 0.0 and 10.0 ± 0.0 mm, respectively). The FAo3 cream gave the highest zone of inhibition (16.0 ± 0.0 mm) on *C. albicans*. The tydineal and mycozoral creams (reference creams) performed better than all the formulated creams.

Table 4 shows the effect of *A. occidentale* creams on mice skin injected with *S. aureus*. Tydineal cream showed the highest bactericidal effect on S. aureus than all the *A. occidentale* creams. Among the formulated creams, FAo2 showed better bactericidal effect than FAo1 and FAo3.
The mice did not display any abnormal behavior during the three weeks application of the *A. occidentale* creams on their skins; and also, there was neither redness nor oedema on their skins when assessed visually. Finally, the photomicrographs of the mice skins show no visible lesions (Plate 1).

Table 4:
Effect of *Anacardium occidentale* creams on mice skin injected with *Staphylococcus aureus*

Parameters	*A. occidentale* (%)	Bacteria survival (%)	Bactericidal effect (%)
FAo1	2.5	14.77	85.23
FAo2	5.0	14.61	85.39
FAo3	10	14.68	85.32
Tydineal cream	NA	12.07	87.93

FAo = cream containing ethyl acetate leaf fraction of *A. occidentale*. NA = Not applicable.



W, X, Y treated with creams having 2.5, 5, 10 % ethyl acetate leaf fraction of *A. occidentale* respectively, Z = Untreated skin (control). Sebaceous glands are clumped together in W; dermal connective tissue is thin, congested and infiltrated in X; very scanty adipose layer in Y; very sparse and loose dermal collagen in Z. All creams show no visible lesions.

Plate 1:
Photomicrographs of mice skins (x 400) treated with *Anacardium occidentale* creams showing no visible lesions

DISCUSSION

The ethyl acetate fraction of *Anacardium occidentale* leaf was formulated into creams, and their physicochemical properties, antimicrobial ability and toxicity on mice skins were evaluated. The colour of the cream was based on the concentration of the *A. occidentale* fraction and its interaction with the base. The humectant, hydrophilic substance that prevents water loss from formulations, added shinning property to the creams. Patients are more compliant to medications that have pleasant colour (Ajala, 2014). The density of the *A. occidentale* creams increased as the concentrations of the plant fraction increased. The mean time to extrude the cream from the tube was highest in FAo1 but lowest in FAo3 as influenced by their densities. Likewise, the mean spreading length was highest in FAo1 while least in FAo3. Normally, cream formulations with humectants spread faster than those without them (Ajala *et al*., 2016). The good spreading values of FAo1, FAo2 and FAo3 creams might be from the base and the humectant. Moreover, their spreading values would dictate their bio-accessibility on the skin.
The FAo2 cream had the highest pH value while FAo1 cream was least. The pH values of the cream formulations remarkably reduced when compared to the pH of the leaf fraction, this was due to the addition of the humectant and the base. Comparatively, the pH of *A. occidentale* leaf fraction (4.92 ± 0.14) was higher than the pH of the cashew juice, nectar and pulp (4.14±0.01, 3.51±0.02, and 3.58±0.04 respectively) reported by Rogeria *et al*. (2018). The pH of the FAo1 cream was closer to that of the nectar but similar to that of the pulp. The pH range for optimal growth of *Staphylococcus aureus* is 7.0 - 7.5, yet it is capable of reproducing at 4.5 - 9.0. The pH value of the normal skin surface, 4.5–6.0, is consequently insufficient to kill the bacteria completely; but slight acidic environment is a disadvantage for bacterial colonization (Kuo *et al*., 2020). Therefore, the pH values of the formulated creams (FAo1, FAo2 and FAo3) are within the safe and satisfactory range with antibacterial advantage.
The FAo1 cream had the highest rate of diffusion while FAo3 cream was least. The humectant (propylene glycol) diluted the leaf fraction, thus helped the diffusion rate of the creams; comparatively, Ajala *et al*. (2016) reported that the presence of humectant (glycerin) facilitated faster diffusion in *Phyllanthus amarus* creams. The FAo2 cream had the largest diameter for the globule size while FAo3 cream was least. Sochorová *et al*. (2017) reported that the outermost layer of skin is the main barrier for penetration of substances. Additionally, the maximum globule size for a conventional cream is 100 µm (Kamath and Sivakumar, 2017).; though, micro-particles with diameters more than 10 µm stay on the skin surface but those less than 3 µm are randomly dispersed into hair follicle and stratum corneum (Ajala *et al*., 2016). The globule sizes of FAo1 and FAo3 creams are less than 100 µm, and this means that they are within the suitable limit.
The viscosities of FAo1 and FAo2 creams were closer and higher than FAo3 cream. Noticeably, the addition of humectant lowers the viscosity of the *A. occidentale* creams, thus making them more fluid. Ajala *et al*. (2016) expatiated that high viscosity value in a formulation shows that the attractive forces between the molecules are high, and thus gives it high yield stress that keeps them from running off the skin surface after application. The high viscosity aids to prolong drug release at the application site. Thus, FAo1, FAo2

and FAo3 creams will stick on the skin surface to deliver their beneficial intention.

The values of viscosity were inversely proportional to that of the rotational speed. Consequently, the rheological profiles of the creams showed non-Newtonian flow pattern that make them to behave as solid on the skin surface until a force is applied to spread them (Simões *et al*., 2020). The rheology of the formulated creams were pseudoplastic in nature and this implies that they will cling as films unto the skin surface and act over a prolonged period until they are washed off. This is an acceptable parameter for semi solid formulations like cream (Ajala *et al*., 2016).

The ethyl acetate fraction of *A. occidentale* leaf was active against all the tested fungi, therefore contradicts the reports of Ajileye *et al*. (2015), that the ethyl acetate fraction of *A. occidentale* leaf has no anti-fungal property. However, the inhibition of *A. occidentale* leaf against *C. albicans* corroborates the report of Shetty *et al*. (2014). It also supports Chan *et al*. (2017) who reported that *A. occidentale* leaf possesses antibacterial and antifungal properties.

The anti-microbial activities of ethyl acetate fraction of *A. occidentale* leaf were better than those of the formulated creams except for *S. aureus* and *S. aureus* ATCC 27853. It might be suggested that the cream base was more compactible and thus enhanced the anti-microbial activity against *S. aureus* and *S. aureus* ATCC 27853, but was indifferent to *P. aeruginosa*, *P. aeruginosa* ATCC 29213 and *Epidermophyton* species, and decreased activities on *T. rubrum* and *C. albicans*. *Staphylococcus aureus* causes infantile-impetigo, breast abscess, carbuncles and boils; *S. aureus* and *P. aeruginosa* cause folliculitis (Ratnam *et al*., 2017). Fungal invasion is limited to the body surface, such as the stratum corneum, hair, nails and the surfaces of mucous membranes of the oral cavity and vulva (Mochizuki *et al*., 2020). Many researchers have reported that *T. rubrum*, *T. mentagrophytes* and *Epidermophyton floccosum* cause tinea barbae, tinea pedis, tinea unguium, tinea cruris and tinea incognito; *T. rubrum* and *T. mentagrophytes* also cause tinea capitis and tinea faciei (Lin *et al*., 2004; Tao-Xiang *et al*., 2005; Starova *et al*., 2010; Sharma *et al*., 2015); however, Lim *et al*. (2023) reported in a study that *T. rubrum* was the main causative fungus of tinea corporis, tinea cruris and tinea pedis while tinea capitis was mainly caused by *Microsporum canis*. Human skin as a vital organ needs serious protection from fungi, bacteria and any microbes causing skin infection diseases (Mlozi *et al*. 2023). Fungal skin infections usually involve itching, skin discoloration, and changes in skin texture in the affected area (Sepahvand, 2018). In treating the problem of fungal infections, Farahmand *et al*. (2016) reported that herbal medicine as an independent treatment way or together with western medicine could be useful in treating fungal infections. Consequently, the formulated creams (FAo1, FAo2 and FAo3) might be incorporated into commercial creams for more effectiveness against bacterial and fungal infections.

Kaenhin and Mungmai (2023) reported that the ethanol extracts from cashew leaves showed promise for use in skincare product development. An ideal tropical product should produce a desired pharmacological effect and has an acceptable safety profile on the skin (Ajala *et al*., 2016). Skin colour, tone and evenness, pigmentation, and skin surface characteristics are signs of skin's health (Mohiuddin, 2019). The formulated creams (FAo1, FAo2 and FAo3) from the ethyl acetate fraction of *A. occidentale* leaf showed no observable lesions on the mice skins and thus might be regarded as a safe topical product.

CONCLUSION

In this investigation, the ethyl acetate fraction of *A. occidentale* leaf has antibacterial and antifungal properties. The formulated creams from the *A. occidentale* leaf have acceptable physicochemical properties, active against the selected infectious microorganisms, safe as topical application and therefore might be further researched, improved and commercialized.

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