JOPAT Vol 23(2), 1607- 1618, July – December, 2024 Edition.
ISSN2636 – 5448 https://dx.doi.org/10.4314/jopat.v23i2.17

Gas Chromatography-Mass Spectrometry Analysis, Druggability and *in-silico* Dermatopharmacokinetics Screening of *Mitracarpus scaber* Extract.

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Abstract

Medicinal plants are an important source of natural compounds used in the development of drugs to treat infectious diseases. The plant *Mitracarpus scaber* has traditionally been used to treat various ailments, including skin disorders. In this study, GC-MS (gas chromatography-mass spectrometry) analysis was used to identify eighteen bioactive components in the methanolic extract of *Mitracarpus scaber* whole plants. To assess the druggability and skin pharmacokinetics of these phytocompounds, in silico screening was performed using online programs such as Swiss ADME, pkCSM, ADMETLab 2.0, and StopTox. The druggability assessment of the identified compounds met the requirements of Lipinski's Rule of Five, which is a set of standards used to estimate the likelihood of good oral drug absorption. The pharmacokinetic factors such as skin sensitization, acute inhalation toxicity, acute dermal toxicity, skin irritation and corrosion, skin permeability, Ames toxicity, carcinogenicity, eye corrosion, eye irritation, and oral acute toxicity were screened. Results indicated that the plant may be safe for therapeutic use since they do not exhibit acute oral toxicity and Ames toxicity and also had less acute dermal toxicity and acute inhalation toxicity. However, direct eye contact with the compounds should be avoided due to its potential to cause eye corrosion and eye irritation. Therefore, this *in-silico* screening method should be encouraged for the preclinical study of medicinal plants to avoid costly mistakes in the course of drug discovery and development

Key words: *In-silico,* pharmacokinetics, druggability, skin and phytocompounds

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Introduction

The discovery and development of medications to treat infectious diseases heavily rely on medicinal plants as their main source of natural lead chemicals [1]. Altaf *et al*. [2] have suggested that the varied array of bioactive chemicals present in these plants account for their effectiveness and treatment value. The process of finding new pharmaceutical drugs, however, is expensive, time-consuming, and rife with danger. Preclinical and clinical studies must be conducted, along with the selection of a particular disease, target identification, lead discovery, lead optimization, and so on [3].

Drugs administered topically to the skin have complex pharmacokinetics that are difficult to quantify. Drug levels in pertinent skin tissues are a primary source of difficulty for experiments attempting to measure them [4]. Frang [5] notes that there is also a lack of popular acknowledgment of the systemic circulation as an appropriate "surrogate" compartment for the skin. There is a concern that the skin pharmacokinetics may not be adequately informed by blood levels because of the inherent challenges in measuring the medication.

However, knowledge of skin pharmacokinetics is necessary for the creation of topical treatments for skin conditions [6]. Effective formulation development and the design of a suitable dosing regimen depend on knowing whether the medication reaches a therapeutic concentration at its targeted site of action, such as the basal epidermis [4].

Mitracarpus scaber is a member of the genus *Mitracarpus*, family Rubiaceae, and species [7]. Many ailments, including headaches, toothaches, amenorrhoea, dyspepsia, hepatic disorders, venereal illnesses, and leprosy, are treated with this plant in West African traditional medicine [7]. Traditionally, the juice is locally used to treat skin conditions like eczema, rabies, and infectious dermatitis [8]. As a result, these conventional uses have prompted scientific research to confirm the claimed medicinal benefits of *M. scaber* [9].

The term "druggability" describes a drug molecule's capacity to be transformed into a medicinal agent [10]. This idea includes things like the safety profile, pharmacokinetics, and chemical characteristics of the medicine. Analysing a drug's absorption, distribution, metabolism, and excretion from the body is the subject of pharmacokinetics [11]. It is easier to understand the drug's behaviour when these factors are understood, including bioavailability, half-life, and clearance.

The efficiency of assessing the pharmacokinetics of medicinal plants can be greatly improved by developing a rapid and easy-to-use process for predicting a large number of chemical constituents and then carrying out in vivo and in vitro pharmacological experiments [12]. Platforms such as Swiss ADME, pkCSM, ADMETLab 2.0, and StopTox websites allow the prediction of pharmacokinetic features, drug-like nature for one or more small compounds [12]. The purpose of this work is to submit the bioactive chemicals identified in *M. scaber* with the aid of GCMS analysis into the websites of Swiss ADME, pkCSM, ADMETLab 2.0, and StopTox for *in-silico* screening of individual phytochemicals to forecast the pharmacokinetic characteristics of the skin.

Materials and Methods

Collection of plants and identification

The whole plant of *M. scaber* was collected from Maikunkele in the Bosso Local Government of Minna, Niger State. The plant was taken to the Herbarium Unit, National Institute of Pharmaceutical Research and Development (NIPRD), Idu, Abuja for authentication. The Voucher specimen was deposited at the Herbarium section for future reference as NIPRD/H/7444.

Preparation of plant material and Extraction

A 100 g amount of the powdered plant was immersed in 600 ml of methanol. The mixtures were agitated every 6 hours while being left to stand at room temperature (28 ±2 ºC) for seven days. The extract was filtered using Whatman (No.1) filter paper after being sieved through muslin cloth [13]. To create a greasy mass, the extracts were concentrated by heating them to 50 °C in a water bath. Once the greasy mass was formed, it was utilized as the final material for the extraction of methanol. It was then placed into screw-cap bottles, labeled, and kept in a refrigerator between 2 and 5°C until needed.

GC-MS Analysis

The GC-MS analysis of the methanol extract of *Mitracarpus scaber* whole plants was carried out using GC-MS (Shimadzu- QP 2010 PLUS, Japan). GC-MS is a combination of two analytical techniques into a single method of analyzing the mixtures of chemical compounds. Gas chromatography separates the components of mixture and mass spectrometry analyses each of the components separately.

Identification of Components

The National Institute of Standards and Technology (NIST) database, which contains over 62,000 patterns, was used to conduct interpretations on the mass spectrum of the GC-MS. The names, molecular weights, and structures of the components of the test materials were determined, and the results were tabulated. The spectra of the unknown and known components were compared [14].

Druggability

The canonical SMILES strings of the 18 bioactive compounds entered into the Swiss ADME web server (www.swissadme.ch) and pkCSM web server (https://biosig.lab.uq.edu.au/pkcsm/prediction) to evaluate their druggability. Lipinski's Rule of Five was applied. According to this rule, if a compound has more than five H-bond donors, ten H-bond acceptors, a molecular weight exceeding 500, and a computed Log P (Log P) higher than five, it may have low solubility and/or poor permeability. Compounds with two or more Lipinski's Rule of Five violations would be considered to have low solubility and/or poor permeability [15].

Results and Discussion

The study of pharmacokinetics (PK) aims to comprehend the way the body responds to substances that are delivered, such as chemicals or medications [16]. PK parameters, such as skin sensitization, skin irritation and corrosion, skin permeability, eye irritation, eye sensitization, acute dermal toxicity, acute inhalation toxicity, acute oral toxicity, and carcinogenicity of 18 compounds from methanolic extracts of *M. scaber*, were studied using the following online free tools: SwissADME web server (www.swissadme.ch) [17] ADMETlab 2.0 web server (https://admetmesh.scbdd.com/service/screening/index) [18], pkCSM web server (https://biosig.lab.uq.edu.au/pkcsm/prediction) [19], and TopTox web server (https://stoptox.mml.unc.edu/) [20]. They were evaluated using canonical SMILES strings as file input from 18 compounds.

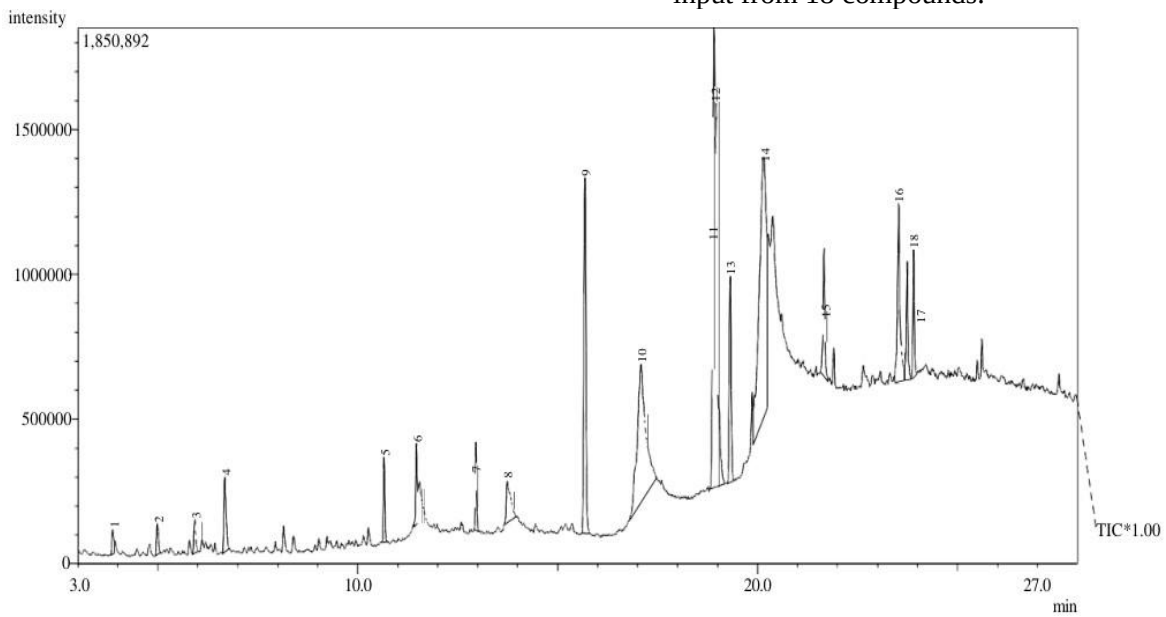

Figure. 1: GC-MS chromatogram of methanol extracts of *Mitracarpus scaber* whole plant

Gas chromatography-mass spectroscopy

The GC-MS investigation of the methanol extract of *M. scaber* whole plant revealed the presence of eighteen different compounds. The chromatogram is shown in Figure. 1, and Table 1 show the list of chemical components in the methanol extracts together with their molecular structures, concentrations (%), names of the compounds, canonical smiles and molecular weights. The compounds were: 3-(Allyloxy)-2-methyl-1-propene (0.61%), Endo-8,9-dihydrodicyclopentadiene (0.77%), Allylidene cyclohexane (0.79%), 4-Phenylbut-3-ene-1-yne (2.08%), Decanoic acid, methyl ester (1.38%),

Decanoic acid, ethyl ester (1.56%), Methyl 15-methyl hexadecanoate (1.44%), Methyl 2,6-dimethyloctanoate (2.33%), Hexadecanoic acid, methyl ester (9.18%), n-Hexadecanoic acid (14.60%), 1,3-dioxolane (13.50%), 11-Octadecenoic acid, methyl ester (10.12 %), Octadecanoic acid, methyl ester (4.58%), Oleic acid (23.22%), Stearic acid methyl ester (3.08%), 4-Trifluoroacetoxy hexadecane (5.97%), 1-(6-Methyl-2-pyridyl)-3-phenylurea (2.70%) and Docosanoic acid, methyl ester (2.19%).

Among these compounds in the methanol extract, eight of them (Oleic acid, n-Hexadecanoic acid, 1, 3-dioxolane, 11-Octadecenoic acid, methyl ester, Hexadecanoic acid, methyl ester, 4-Trifluoroacetoxy hexadecane, Octadecanoic acid, methyl ester and Stearic acid methyl ester) with the highest abundance range between 3.08% - 23.22% were reported to have biological activities [21-26]. The report of biological activities of the some of the major compounds found in *M. scaber* justify its therapeutics purpose in ethnomedicine.

Table 1: Gas chromatography-mass spectroscopy

S/N	Molecular Structure	Area %	Name of the Compounds	Canonical smiles	Molecular Weight
1	C_7H_{120}	0.61	3-(Allyloxy)-2-methyl-1-propene	CC(=C)COCC=C	112
2	$C_{10}H_{14}$	0.77	Endo-8,9-dihydrodicyclopentadiene	C1CC2CC1C3C2C=CC3	134
3	C9H14	0.79	Allylidenecyclohexane	C=CC=C1CCCCC1	122
4	$C_{10}H_8$	2.08	4-Phenylbut-3-ene-1-yne	C#CC=CC1=CC=CC=C1	128
5	$C_{11}H_{22}O_2$	1.38	Decanoic acid, methyl ester	COC(CCCCCCCCC)=O	186
6	$C_{12}H_{24}O_2$	1.56	Decanoic acid, ethyl ester	CCCCCCCCCC(=O)OCC	200
7	$C_{18}H_{36}O_2$	1.44	Methyl 15-methylhexadecanoate	CC(C)CCCCCCCCCCCCCC(=O)OC	285
8	$C_{11}H_{22}O_2$	2.33	Methyl 2,6-dimethyloctanoate	CCC(C)CCCC(C)C(=O)OC	186
9	$C_{17}H_{34}O_2$	9.18	Hexadecanoic acid, methyl ester	O=C(OC)CCCCCCCCCCCCCCC	270
10	$C_{16}H_{32}O_2$	14.60	n-Hexadecanoic acid	CCCCCCCCCCCCCCCC(=O)O	256
11	$C_3H_6O_2$	13.50	1,3-dioxolane	C1COCO1	74
12	$C_{19}H_{36}O_2$	10.12	11-Octadecenoic acid, methyl ester	CCCCCCC=CCCCCCCCCC(=O)OC	296
13	$C_{19}H_{38}O_2$	4.58	Octadecanoic acid, methyl ester	CC=CCCCCCCCCCCCCCC(=O)OC	298

14	$C_{18}H_{34}O_2$	23.22	Oleic Acid	CCCCCCCCC=CCCCCCCC(=O)O	282
15	$C_{19}H_{38}O_2$	3.08	Stearic acid methyl ester	CCCCCCCCCCCCCCCCCC(=O)OC	298.5
16	$C_{18}H_{33}F_3O_2$	5.97	4-Trifluoroacetoxy hexadecane	CCCCCCCCCCCCCC(CCC)OC(=O)C(F)(F)F	338.4
17	$C_{13}H_{13}N_3O$	2.70	1-(6-Methyl-2-pyridyl)-3-phenylurea	CC1=NC(=CC=C1)NC(=O)NC2=CC=CC=C2	227
18	$C_{23}H_{46}O_2$	2.19	Docosanoic acid, methyl ester	CCCCCCCCCCCCCCCCCCCCCC(=O)OC	354

Druggability Characteristics of the Phytocompounds

The druggability behavior of eighteen phytocompounds present in the methanolic extract of *M. scaber* whole plant was predicted in Table 2. The molecular weight ranged from 74 to 358 grams per mole. LogP values ranged from -0.0 to 7.9. Nine of eighteen compounds had one violation each for logP value. The number of hydrogen bond acceptors ranged from 0 to 2 kJ/mol. The number of hydrogen bonds ranged from 0 to 2 kJ/mol. All the compounds passed the drug-likeness test as regards oral drug absorption. According to Lipinski's rule of five, if any compound had two or more Ro5 violations, it would have low solubility and/or poor permeability [15].

Table 2: The Result of Computed Lipinski's Rule of Five (Ro5)

Bioactive Compounds	TEST					
	Molecular weight	LogP Value	Number of Hb acceptor	Number Hb donor	Lipinski violation	Druggability Remark
3-(Allyloxy)-2-methyl-1-propene	112	1.8	1	0	No V	Pass
Endo-8,9-dihydrodicyclopentadiene	134	2.6	0	0	No V	Pass
Allylidenecyclohexane	122	3.1	0	0	No V	Pass
4-Phenylbut-3-ene-1-yne	128	2.3	0	0	No V	Pass
Decanoic acid, methyl ester	186	3.3	2	0	No V	Pass
Decanoic acid, ethyl ester	200	3.7	2	0	No V	Pass

Methyl 15-methylhexadecanoate	284	5.9	2	0	1 V	Pass
Methyl 2,6-dimethyloctanoate	186	3.0	2	0	No V	Pass
Hexadecanoic acid, methyl ester	270	5.6	2	0	1 V	Pass
n-Hexadecanoic acid	256	5.6	1	1	1 V	Pass
1,3-dioxolane	74	-0.0	2	1	No V	Pass
11-Octadecenoic acid, methyl ester	296	6.2	2	0	1 V	Pass
Octadecanoic acid, methyl ester	298	6.2	2	9	1 V	Pass
Oleic Acid	282	6.1	1	1	1 V	Pass
Stearic acid methyl ester	298.5	6.4	2	0	1 V	Pass
4-Trifluoroacetoxy hexadecane	338	6.6	2	0	I V	Pass
1-(6-Methyl-2-pyridyl)-3-phenylurea	227	3.0	2	2	No V	Pass
Docosanoic acid, methyl ester	358	7.9	2	0	1 V	Pass

Key: Hb Hydrogen bond, V violation

***in-silico* Dermatopharmacokinetic Screening**

Quantitatively characterizing the local pharmacokinetics of topically applied medicines in the skin has proven to be challenging because it is difficult to experimentally detect medication levels in the relevant skin tissues [4]. However, using online ADMET tools (pkCSM, SwissADMET, ADMETLab 2.0, and STOptox), the following pharmacokinetic parameters such as skin sensitization, acute inhalation toxicity, acute dermal toxicity, skin irrigation & corrosion, skin permeability, Ames toxicity, carcinogenicity, eye corrosion, eye irritation, and oral acute toxicity have been evaluated for the 18 compounds in methanolic extracts of Mitracarpus scaber in Table 3. The result of skin permeability from pkCSM's online bioinformatics prediction showed that seven compounds (3-(Allyloxy)-2-methyl-1-propene, Endo-8,9-dihydrodicyclopentadiene, Allylidene cyclohexane, 4-Phenylbut-3-ene-1-yne, Decanoic acid, methyl ester, Decanoic acid, ethyl ester, Methyl 2,6-dimethyloctanoate) had high skin permeability, while the remaining eleven had low skin permeability. Skin permeability (Kp) defines the rate at which a chemical penetrates across the stratum corneum [27]. Kp is considered in the development of transdermal drug delivery [28].

Percentage of absence (No) and presence (Yes) of the rest of the predicted compounds' pharmacokinetics, which gave a quantitative idea of the toxicity of the extract, were further shown in Table 4. The Ames toxicity test, which measures the ability of any substance to cause mutation [29], and oral acute toxicity, which

refers to adverse effects occurring following oral administration of a single dose of a substance [30], were 100% absent in the methanol extract. This was followed by acute inhalation toxicity (88.9%) and acute dermal toxicity (88.9%). Adverse symptoms that arise after four hours of inhalation exposure to gas, dust, mist, or vapour are referred to as acute inhalation toxicity [31]. When a chemical is applied topically, either once or more times in a 24-hour period, the term "acute dermal toxicity" refers to the unfavourable consequences that manifest instantly [32].

The percentages of absence for carcinogenicity, skin irritation, and corrosion were 66.7% and 55.6%, respectively. The carcinogenic mechanism of chemicals may be due to their ability to damage the genome or disrupt cellular metabolic processes [33]. Because carcinogenicity has such detrimental impacts on human health, it is a critical issue. Skin corrosion is described as apparent, irreversible necrosis from the epidermis to the dermis brought on by chemical exposure to the skin [34]. Skin irritation is defined as restricted, reversible damage caused by chemical contact to the skin.

The presence of eye irritation and eye corrosion were 100% and 94.4%, respectively, followed by skin sensitization (88.3%). Eye irritation is a term for eye discomfort, itchiness, or dryness [35].

Eye corrosion occurs when a test material is applied to the front surface of the eye, resulting in either significant physical degradation of eyesight or damage to the eye's tissue [36]. A delayed T-cell-mediated allergic response to chemically altered skin proteins is known as skin sensitization, and it is a reaction of the adaptive immune system [37].

Table 3: The Result of *in-silico* Dermatopharmacokinetics Screening Predicted

Bioactive Compounds	TEST									
	SS	AIT	ADT	SIC &	SP	AT	Ca	EC	EI	OAT
3-(Allyloxy)-2-methyl-1-propene	Yes	Yes	No	Yes	High (-2.025)	No	Yes	Yes	Yes	No
Endo-8,9-dihydrodicyclo Pentadiene	No	No	Yes	Yes	High (-1.701)	No	No	Yes	Yes	No
Allylidenecyclohexane	Yes	No	No	Yes	High (-1.14)	No	Yes	Yes	Yes	No
4-Phenylbut-3-ene-1-yne	Yes	No	Yes	Yes	High (-1.073)	No	Yes	Yes	Yes	No
Decanoic acid, methyl ester	Yes	No	No	No	High (-1.643)	No	No	Yes	Yes	No
Decanoic acid, ethyl ester	Yes	No	No	No	High (-1.697)	No	No	Yes	Yes	No
Methyl15-methyl hexadecanoate	Yes	No	No	No	Low (-2.683)	No	No	Yes	Yes	No
Methyl 2,6-dimethyloctanoate	Yes	No	No	No	High (-1.722)	No	No	Yes	Yes	No

Hexadecanoic acid, methyl ester	Yes	No	No	No	Low (-2.528)	No	No	Yes	Yes	No
n-Hexadecanoic acid	Yes	No	No	Yes	Low (-2.717)	No	No	Yes	Yes	No
1,3-dioxolane	No	No	No	No	Low (-3.04)	No	Yes	Yes	Yes	No
11-Octadecenoic acid, methyl ester	Yes	No	No	Yes	Low (-2.28)	No	No	Yes	Yes	No
Octadecanoic acid, methyl ester	Yes	No	No	No	Low (-2.758)	No	No	Yes	Yes	No
Oleic Acid	Yes	No	No	Yes	Low (-2.725	No	No	Yes	Yes	No
Stearic acid methyl ester	Yes	No	No	No	Low (-2.792)	No	No	Yes	Yes	No
4-Trifluoroacetoxy hexadecane	Yes	Yes	No	Yes	Low (-2.697)	No	Yes	Yes	Yes	No
1-(6-Methyl-2-pyridyl)-3-phenylurea	No	No	No	No	Low (-3.279)	No	Yes	No	Yes	No
Docosanoic acid, methyl ester	Yes	No	No	No	Low (-2.825)	No	No	Yes	Yes	No

Keys: SS skin sensitization, AIT acute inhalation toxicity, ADT acute dermal toxicity, SI & C skin irrigation & corrosion, SP skin permeability, AT Ames toxicity, Ca carcinogenicity, EC eye corrosion, EI eye irritation, OAT, oral acute toxicity, No absent, yes present

Table 4: The Result of *in-silico* Dermatopharmacokinetic Screening Predicted by Percentage

S/N	Pharmacokinetics parameters Screened	Absent (%)	Present (%)
1	Skin sensitization (SS)	3 (16.7%)	15 (83.3%)
2	Acute inhalation toxicity (AIT)	16 (88.9%)	2 (11.1%)
3	Acute Dermal Toxicity (ADT)	16 (88.9%)	2 (11.1%)
4	Ames toxicity (AT)	18 (100%)	0 (0%)
5	Carcinogenicity (Ca)	12 (66.7%)	6 (33.3%)
6	Skin irritation and corrosion (SI&C)	10 (55.6%)	8 (44.4%)
7	Eye corrosion (EC)	1 (5.6%)	17 (94.4%)
8	Eye irritation (EI)	0 (0%)	18 (100%)
9	Oral acute toxicity (OAT)	!8 (100%)	0 (0%)

Key: Absent (%) denote quantitative value toxicity lacking in the entire Methanolic extract of *Mitracarpus scaber* whole plant
Present (%) denote quantitative value toxicity available in the entire Methanolic extract of *Mitracarpus scaber* whole plant

Conclusion

In conclusion, the study of *Mitracarpus scaber*'s bioactive compounds using gas chromatography-mass spectrometry analysis and *in-silico* screening method to assess the druggability and dermatopharmacokinetics provides important new information about the potential therapeutic benefits this plant might have for skin-related ailments. The compounds found throughout this investigation showed very promising druggability qualities, in addition to a very good degree of safety across several pharmacokinetic metrics. However, most of the compounds showed potential toxicity against the eyes, such as eye corrosion and irritation. These noteworthy results provide a strong basis for future study and development efforts concerning topical treatments derived from this medicinal plant. They also significantly contribute to the scientific validation of the traditional application of *Mitracarpus scaber*. Therefore, this *in-silico* method should be encouraged for preclinical study of medicinal plants to avoid costly mistakes in the course of drug discovery and development.

Conflict of Interest

The authors claim no conflict interest

Acknowledgement

The authors wish to thank IBR-TETFUND through Federal University of Technology, Minna for making funds available for this research. We solicit for more funding in other to carry out more aspects of this research especially the production of antimicrobiall cream.

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