



Antidermatophyte Activity of Ethanol Extract and Stability of Creams Formulated from *Cassia alata* Linn

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Authors' contributions

This work was carried out in collaboration among all authors. Author FOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ABF and HL managed the analyses of the study. Author ABF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

There are scientific reports of medicinal plants having curative properties. These properties may be due to the presence of various phytochemicals in the plants. *Cassia alata* is a specie in the *Fabaceae* family. The purpose of this study is to determine the antidermatophyte activity of the extract, formulate a cream with the extract and determine the stability of the cream by measuring the pH, Free-thaw test, Centrifugation and sensitivity to light. We also subjected the formulated cream to temperature variation test at -10, 4, 30, 37 and 45° C. The percentage yield was 8.5%. The extract contain alkaloid (4.24±0.24%), saponin(1.35±0.39%), tannin (0.45±0.29%) and flavonoid (2.42±0.32%) respectively. The antifungal test results showed the activity against the dermatophytes to be in the increasing order *M.furur* (12± 0.2 mm) < *Microsporum audounii* (12±0.4 mm) < *Trichophyton mentagrophtes* (14±0.2mm) < *Epidermophyton floccosum* (16±0.2 mm). Temperature stability and Centrifuge testing indicated that the formulations were stable. Light testing indicated no change in the colour of the cream. Our study showed that the plant

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has high potential as an anti-dermatophyte when formulated as a cream for topical use. The stability of the cream formulations indicated that it can be used for the management of dermatophytosis.

Keywords: *Cassia alata*; centrifugation; antidermatophyte; cream; phytochemicals.

1. INTRODUCTION

Over the years, plant materials have been used to prevent and treat various diseases and this has successfully attracted the attention of scientist's worldwide [1]. Antimicrobial compounds found in plants have great therapeutic potential as they can serve their purpose without any side effects often associated with synthetic drugs and it has also been reported that there is little chance of development of resistance [2]. The resistance to some of the antifungal agents coupled with the high costs of treatments has necessitated the search for new, safe, efficient and cost effective ways for the management of infections [3]. Through the information supplied by the local populations, many investigations are being conducted on medicinal plants with the aim of finding out phytochemical constituents for application in the prevention and management of infections and other diseases of non-microbial etiology [4]. Several studies have been conducted to provide scientific basis for the efficacy of plants in herbal medicines [5]. Scientists such as Akinpelu and Onakaya [1] warned that very soon the population of bacteria developing resistance will not match the arsenal to fight unless concerted efforts are made to acquire new agents,. The rising interests in products of natural origin in the developed economics led to the extraction and development of several drugs and chemotherapeutic agents from plants as well as from traditionally used rural herbal remedies [2].

The medicinal usefulness of *Cassia alata* (Linn) has been the object of many chemical and pharmacological studies. It is an ornamental shrub or tree growing up to 12 m high and widely available in the tropics around towns and villages throughout West Africa [6]. *Cassia alata* leaves are well known for their application in the treatment of fungal infections. This necessitated Abubaker [7] and his co-workers in 2008 to investigate the antifungal activity of the aqueous flower extract of the plant against three distinct groups of fungi which are aflatoxin producing fungi, plant pathogenic fungi and human pathogenic fungi. In their study, it was concluded that the extract can be used as a potential

antifungal agent for these three groups of fungi. Mohammed et al. [5] isolated three anthraquinones (rhein, emodin and aloemodin) from Chloroform extract of *Cassia alata* leaves. These phytochemicals are all known to have useful bioactivities including anticancer activities. Makinde et al. [8] evaluated antibacterial and antifungal activity of the aqueous and methanol extract of *Cassia alata* leaves. In their studies, the extract of the plant was more active against fungi than the bacteria with 20 – 30 mm diameter of zones of inhibition. Preliminary investigation on the phytochemistry and antimicrobial activity of *Cassia alata* leaves was carried out by Idu et al. [9]. It was reported that the leaves contained chrysoeriol, kaempferol, quercetin, 5,7,4'-trihydroflavanone, kaempferol-3-O-beta-D-glucopyranosyl - (1,6) - beta - D-glucopyranoside, 17-hydrotetracontane, n-dotriacontanol, n-triacontanol, palmitic acid, ceryl ester, rutin , stearic acid and palmitic acid. Ethanolic extracts of the leaves of *Cassia alata*, *Baphia nitida*, *Ficus exasperate* and *Gossypium arboreum* were analyzed by Okafor et al. [10], for their antifungal activities against four pathogenic fungi namely: *Trichophyton rubrum*, *Epidermophyton floccosum*, *Basidiobolus haptosporus* and *Candida albicans*. The extracts inhibited the growth of the fungal pathogens except that of *Basidiobolus haptosporus*.

Dermatophytosis is a skin disease caused by fungi commonly called dermatophytes. The infection usually cause hair loss in the area affected. It also makes the skin to appear red, itchy and scaly, showing a circular rash. It is estimated that between 13.8 and 20% of the population has had a dermatophyte infection [11]. It is often successfully treated with topical antifungal agents. However, if not properly treated, these infections may become chronic, requiring oral antifungal drugs, which are often associated with hepatotoxicity [11].

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Leaves of *Cassia alata* were collected at the Botanical Gardens of the University of Ibadan. It was identified and sample deposited with

herbarium number MPNH/2017/1257 at Medicinal Plants of Nigeria Herbarium of NNMDA.

2.2 Preparation of Plant Extracts

The freshly collected leaves were air-dried under shade at room temperature for 7 - 14 days. Upon drying, the plant materials were pounded using mortar and pestle into smaller particles and then subjected to fine powder using an electric blender (Kenwood). The powdered samples were stored in airtight containers and kept at room temperature until required.

2.3 Extraction of Crude Extract

The air-dried powdered plant samples (10.0 g) was soaked in 100 ml of ethanol in a 500 ml sterile conical flasks. The flask was covered with cotton wool plug and then wrapped with aluminium foil and shaken vigorously at 3 h intervals for 48 h at room temperature. The crude extract was then filtered using muslin cloth and then Whatman no. 1 filter paper. The filtrate was evaporated to dryness and the dried substance stored in airtight bottles until required.

2.4 Determination of Phytochemical Constituents

The extract was evaluated for the presence of Tannins, flavonoids, saponins and alkaloids using simple qualitative and quantitative methods of Trease and Evans [12] and Sofowora [13].

2.5 Test Organisms

The test organisms used were clinical isolates of *Microsporium aoudininn*, *Epidermophyton floccosum*, *Trychophyton mentagrophyte* and *Malassezia furfur*. They were obtained from Spectralab Medical and Diagnostic Services, Sagamu, Ogun State.

2.6 Formulation of Creams

Oil in water cream was produced. Oil soluble substances were heated to between 70–75°C. Water-soluble substances were also heated to the same temperature. The oil phase and the aqueous phase were slowly stirred together. The coarse emulsion formed was cooled to about 35°C. The emulsion was allowed to stay at room temperature for twelve hours and then homogenized with the aid of a mechanical stirrer. Three different formularies containing 0.5%, 1%

and 2%, of *Cassia alata* ethanolic extracts were produced. The prepared herbal creams were then vigorously homogenized.

2.7 Physical Evaluation of the Formulations

The cream formulations were visually inspected for their colour, homogeneity, consistency and phase separation.

2.8 Measurement of pH

This was determined using pH Meter Model 290 Mk 2 in the Laboratory of the Nigeria Natural Medicine Development Agency, Lagos. The pH meter was calibrated with buffer solutions 4 and 7 after which the sample solution pH was then taken.

2.9 Stability Tests for the Formulated Creams

Stability tests were carried out on the emulsions following standard methods whereby the temperature variation tests include storing the samples at -10°C, 4°C, 30°C, 37°C and 45°C. The formulated creams were subjected to Freeze thaw cycles testing which involves making the samples pass through three cycles of temperature at -10°C for 24 hours and then at room temperature for 24 hours. The creams were subjected to centrifuge testing whereby the samples were heated to 50°C and then centrifuged for thirty minutes at 2000, 2500, 3000 and 4000 rpm. They were inspected for signs to determine if the dispersed phase of the emulsion has separated and risen to the top. Light testing was also carried out whereby the creams were placed in test tubes and also in the actual package and then put in the window where direct sun rays fell on them. This method is used to determine the sensitivity of the emulsions to the Ultra Violet radiation.

2.10 Microbiological Assay

Zones of inhibition was determined using the method as described by Irobi et al. [14]. The zones of inhibition were measured in mm and recorded.

2.11 FTIR Analysis

The cream base sample and the products were qualitatively characterized by the FT-IR technique.

3. RESULTS AND DISCUSSION

Percentage yield of extracts was 8.5%. The Phytochemical Composition of the Plant was alkaloid (4.24±0.24%), Saponin (1.35±0.39%), Flavonoid (0.45±0.29%) and Tannin (2.42±0.32%).

3.1 Determination of Microbial Activity against the Dermatophytes

The results of the formulations against the dermatophytes are presented in Table 1. It shows 0.5% formulation was most active against *Epidermophyton floccosum* while the activity was same for both *Trychophyton mentagrophyte* and *Microsporium aoudininn*. It had least activity against *Malassezia furfur*. 1% formulation was most active against *Epidermophyton floccosum*. However, the activity was less in *Microsporium aoudininn* and *Trychophyton mentagrophyte*. The least activity was against *Malassezia furfur*. The greatest activity of 2% formulation was against *Epidermophyton floccosum* followed by *Trychophyton mentagrophyte*. The activity was same for both *Microsporium aoudininn* and *Malassezia furfur*. Evidently, the formulations were active against the dermatophytes and it was observed that the activity was concentration dependent with the highest activity against *Epidermophyton floccosum* and the least activity against *Malassezia furfur*.

3.2 Temperature Stability Testing

Table 2 shows the results of determining the pH and change in colour of the formulations while under the accelerated stability test. It was observed that there was no change in colour of the formulations from the date of production through 16th week of accelerated stability test. The pH of the formulations remain the same at the low temperature of -10 and 4°C, this indicated that there is restrictions on the movement of ions within the molecular system. However at 30°C, a slight decrease in the pH of 0.5% formulations from 7.20 at production to 7.17 at the end of 16th week was observed. The same observation was made for both 1% and 2% formulations with decrease in pH from 7.20 to 7.16. For samples stored at 37 and 45°C, there were decrease in pH from 7.20 from the date of production to 7.16 and 7.14 respectively at the

end of 16th week of accelerated stability test. There was also no noticeable change in colour. These results show that the formulations were very stable at the various test temperatures.

3.3 Freeze-Thaw Testing

When the formulations were subjected to cycle testing, it was observed that there was no change in all the test samples indicating that putting the products to extreme temperature swings will have no effect on them and they will be stable Table 3.

3.4 Centrifuge Testing

Subjecting the formulations to centrifugation test showed no phase separation at 2000, 2500, 3000 and 4,000 rpm as indicated in Table 4. This indicated that there is proper particulation of the dispersed phase in the continuous phase thereby making the formulations to be stable.

3.5 Light Testing

When the formulations were exposed to UV light, it was observed that they were not sensitive to the UV light (Table 5).

3.6 Fourier Transform Infra-red Spectroscopy Analysis

Results of FTIR spectroscopic studies revealed the presence of various functional groups in the cream and the test samples. As shown in the infra red spectrum of the placebo Fig. 1, there is strong O-H stretch at 3373.00 cm⁻¹ of amines. N=H bending at 1640.00 cm⁻¹ indicating that the placebo contains an unsaturated amide group. O-H bending of phenyl group at 1409.00 cm⁻¹ indicated the presence of carboxylic acid group in the sample, S=O stretch at 1040.00 cm⁻¹ indicated presence of sulphoxide. The Ca cream contains S-S stretch at 470.00 cm⁻¹ indicating presence of polysulphides Fig. 2 . N=H bend at 1637.00 cm⁻¹ shows presence of amine group. The spectrum also shows NH stretch of aliphatic amine at 3376.00 cm⁻¹. The spectrum also shows presence of S=O stretch at 1040.00 cm⁻¹. The presence of some functional groups found in the herbal cream formulations maybe responsible for the plant effectiveness in the management of dermatophytosis.

Table 1. Effect of formulations against the dermatophytes

Formulation in %	0.5	1.0	2.0
<i>Trychophyton mentagrophyte</i>	7 ± 0.4	9± 0.2	14± 0.2
<i>Microsporium aoudininn</i>	7± 0.5	10± 0.2	12± 0.4
<i>Epidermophyton floccosum</i>	8± 0.6	12± 0.3	16± 0.2
<i>Malassezia furfur</i>	6± 0.6	8± 0.1	12± 0.2

Each value is the mean of three measurement ± std in mm

Table 2. Temperature stability testing

Formulations		Day 1	2 weeks	4 weeks	8 weeks	12 weeks	16 weeks
		pH Colour	pH Colour	pH Colour	pH Colour	pH Colour	pH Colour
-10°C	Control	7.02 NCC	7.02 NCC	7.02 NCC	7.02 NCC	7.02 NCC	7.02 NCC
	0.5%	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC
	1.0%	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC
	2.0%	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC
4°C	0.5%	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC
	1.0%	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC
	2.0%	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC	7.20 NCC
30°C	0.5%	7.20 NCC	7.18 NCC	7.18 NCC	7.17 NCC	7.17 NCC	7.17 NCC
	1.0%	7.20 NCC	7.17 NCC	7.16 NCC	7.16 NCC	7.16 NCC	7.16 NCC
	2.0%	7.20 NCC	7.17 NCC	7.16 NCC	7.15 NCC	7.15 NCC	7.16 NCC
37°C	0.5%	7.20 NCC	7.17 NCC	7.17 NCC	7.16 NCC	7.16 NCC	7.16 NCC
	1.0%	7.20 NCC	7.17 NCC	7.17 NCC	7.16 NCC	7.16 NCC	7.15 NCC
	2.0%	7.20 NCC	7.17 NCC	7.17 NCC	7.16 NCC	7.16 NCC	7.16 NCC
45°C	0.5%	7.20 NCC	7.15 NCC	7.15 NCC	7.14 NCC	7.14 NCC	7.14 NCC
	1.0%	7.20 NCC	7.15 NCC	7.15 NCC	7.14 NCC	7.14 NCC	7.14 NCC
	2.0%	7.20 NCC	7.15 NCC	7.14 NCC	7.14 NCC	7.14 NCC	7.14 NCC

No change in colour = NCC, CC = Change in colour

Table 3. Freeze-thaw testing

Control	NPS
0.5% Cream	NPS
1.0% Cream	NPS
2.0% Cream	NPS

NPS= No Phase separation, PS= Phase separation

Table 4. Centrifuge testing

Sample	2000 rpm	2500 rpm	3000 rpm	4000 rpm
Control	NPS	NPS	NPS	NPS
0.5% Cream	NPS	NPS	NPS	NPS
1.0% Cream	NPS	NPS </td <td>NPS</td> <td>NPS</td>	NPS	NPS
2.0% Cream	NPS	NPS	NPS	NPS

NPS= No Phase separation, PS= Phase separation

Table 5. Light Testing

Control	NCC
0.5% Cream	NCC
1.0% Cream	NCC
2.0% Cream	NCC

NCC =No colour change, CC= colour change

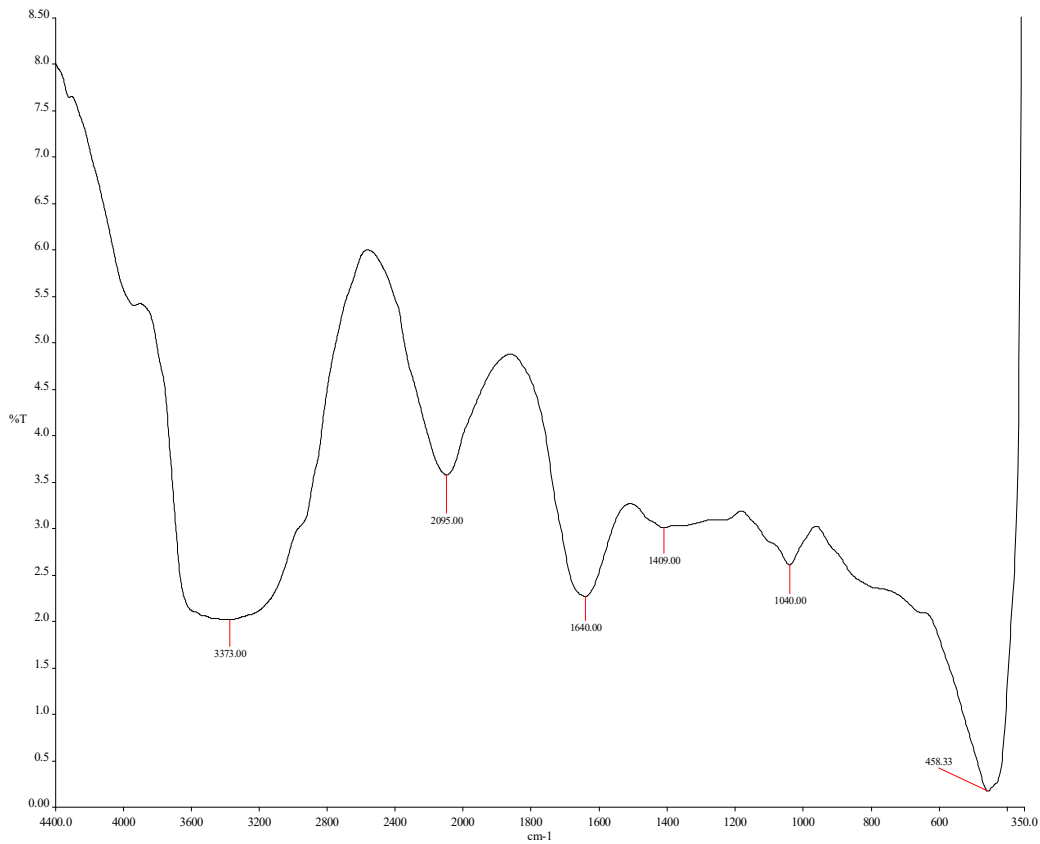


Fig. 1. FTIR spectrum of emulsion alone (placebo)

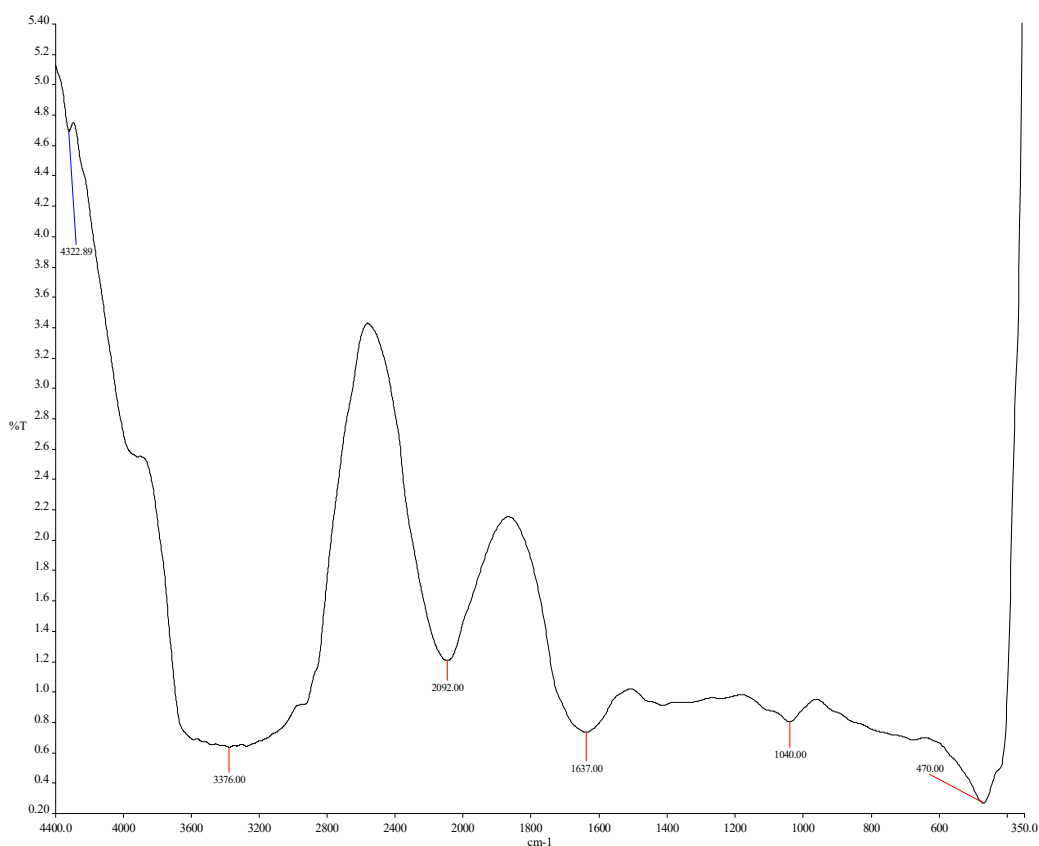


Fig. 2. FTIR spectrum of *Cassia alata* cosmeceutical

4. CONCLUSION

This study shows that *Cassia alata* cream formulation has high potential as antidermatophyte agent which can be used for topical application on the skin. This also explain the successes claimed in the folk use of the plant in the management of skin infections. The 2% cream formulation showed highest activity against all the test microorganisms. It was observed that all the formulations showed acceptable physical properties and were stable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Akinpelu DA, Onakaya TM. Antimicrobial activities of medicinal plantsused in folklore remedies in South- Western Nigeria. Afr. J. Biotechnol. 2006;5(11): 1078-1081.
2. UNESCO. FIT/504-RAF-48. Terminal report. Promotion of ethnobotany and the sustainable use of plant resources in Africa, Paris. 1998;60-61.
3. Gupta AK, Cooper EA. Update in antifungal therapy of Dermatophytosis. Mycopathologia. 2008;166:353–67.

4. Falodun A, Okenroba LO, Uzoamaka N. Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of *Euphorbia heterophylla* Linn (Euphorbiaceae). Afr. J. Biotechnol. 2006;5(6):529-531.
5. Mohammed I. Ali, Ahmed M. Aboul-Enein, Samy M. Mohamed, Faten M. Abouelella, Magdy MD Mohammed, Ahmed R Hamed. Phytochemical, cytotoxicity and antioxidant investigation of *Cassia alata* leaves growing in Egypt. Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS) ISSN. 2015;2349-2759.
6. El-Mahmood AM, Doughari JH. Phytochemical screening and antibacterial evaluation of the leaf and root extracts of *Cassia alata* Linn African Journal of Pharmacy and Pharmacology 2008;2(7): 124-129.
7. Abubacker MN, Ramanathan R, Kumar ST. Natural Product Radiance. 2008;7(1):6-9.
8. Makinde AA, Igoli JO, Ta'Ama L, Shaibu SJ. African Journal of Biotechnology. 2007;6(13):1509-1510.
9. Idu M, Oronsaye FE, Igeleke CL, Omonigho SE. Preliminary investigation on the phytochemistry and antimicrobial activity of *Cassia alata* leaves. Journal of Applied Sciences. 2006;6(11):2481-2485.
10. Okafor JC. Tropical plants in health care delivery in Nigeria. Journal of College of Medicine. 2001;7(2):129-131.
11. Benli M, Bingol U, Geven F, Guney K, Yigit N. An investigation on to antimicrobial activity of some endemic plant species from Turkey. Afr. J. Biotechnol. 2008; 7(1):001-005.
12. Trease GE, Evans WC. Pharmacognosy. 11th Edn. Brailliar Tiridel Can. Macmillian Publishers; 1989.
13. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria. 1993;289.
14. Irobi ON, Daramola SO, Tasie SB, Oyekke SB, Tsado ML. Bacterial properties of crude extracts of *Mitracarpus villos* L (SW) D. C (Synonym *Mitracarpus scaber* Zucc.) Rubiaceae. Nigerian Journal of Microbiology. 2006;9:9-12.

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