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COMPARATIVE STUDIES ON THE ACTIVITY OF EXTRACTS AND STABILITY OF ANTIDERMATOPHYTE CREAMS FORMULATED FROM *CASSIA OCCIDENTALIS LEAVES* Linn

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ABSTRACT

Leaves of *Cassia occidentalis* L were extracted with water, ethanol and petroleum ether. They were then screened for their activities against some selected dermatophytes. Creams formulated with the extracts of the leaves were subjected to stability tests by measuring the pH, Free-thaw test, Centrifugation and sensitivity to light. The formulated creams were subjected to temperature variation test at -10, 4, 30, 37 and 45°C. Ethanol and aqueous extracts showed significant antidermatophyte activity against most of the tested dermatophytes but the most active is the petroleum ether extract against *Trichophyton mentagrophtes* (12±1mm) followed by *Microsporum audounii* and *Epidermophyton floccosum* (12±0mm) and lastly by Malassezia *furfur* (10± 0 mm). All the formulations were stable to Temperature variation. However, petroleum ether extract based cream coalescence during Centrifuge testing at 4000rpm. There was no change in the colour of the creams during light testing. Moisture loss on drying indicated Co.E (6.36%) < Co.W (6.39%) < Co.Pet (6.41%). From the result obtained, it showed that the plant extracts can be utilized in the management of dermatophytosis when formulated as a cream for topical use.

KEYWORDS: *Cassia occidentalis*, Antidermatophyte, Cream, Centrifugation, Stability.

INTRODUCTION

Over the centuries, there has been tremendous progress in human medicines against infectious diseases caused by bacteria, fungi, viruses and other human pathogens. Unfortunately, these pathogens are still a major threat to public health. The impact is mostly felt in developing countries of the world due to relative unavailability of medicines and the emergence of widespread drug resistance.[1] The last few decades has witnessed the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics,[2] This has lead to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages.[3] New research now focuses on natural molecules and products primarily on plants since they are readily available and can be sourced locally based on their ethno-medicinal uses.[2]

Cassia occidentalis L belongs to family *Caesalpiniaceae*.[4] reported that the plant was used in India against various skin diseases such as ringworm, eczema, and scabies due to the high incidence of skin diseases, especially among the weaker section of the Indian population. Various pharmacological investigation by scientists revealed that the plant have antibacterial.[5,6] antimalarial,[7] antimutagenic,[8] antimutagenic,[9] antiplasmodial,[10] anticarcinogenic,[9] and hepatoprotective,[1] Moreover, studies on this plant showed that the nature and amount of the phytochemicals varies according to the season and geographical location.[11] The main phytochemicals chemicals in *C. occidentalis* include: achrosine, emodin, anthraquinones, anthrones, apigenin, sitosterols, tannins and xanthones.[12]

Phytochemical analysis of the aqueous extracts of *Cassia occidentalis* leaf by,[13] indicated that the leaves contained tannins, anthraquinones, sterols, glycosides, saponins and alkaloids, all biochemicals needed for treating skin related diseases. Vedpriya *et al.*,[14] screened different organic and aqueous extracts of leaves of *Cassia occidentalis* for their antimicrobial activity against seven human pathogenic bacteria and two fungal strains by disk diffusion assay. Among these extracts, methanol and aqueous extracts showed significant antimicrobial activity against most of the tested microbes. The most susceptible microorganism was *P. aeruginosa* (18 mm zone of inhibition in aqueous extract) followed by *P. mirabilis* (15 mm) zone of inhibition in methanol extract) and *Candida albicans* (8mm zone of inhibition in methanol extract). The

presence of anthraquinones, carbohydrates, glycosides, cardiac glycosides, steroids, flavanoids, saponins, phytosterols, gums and mucilages were confirmed, while alkaloids were absent in all the tested extracts.

The phytochemical screening of petroleum ether, chloroform and methanolic extracts of *Cassia occidentalis* were performed by Muyibi *et al.*,[15] The chloroform and methanolic extracts of both flower and seed were found to contain flavonoids, alkaloids, phenolics tannins, steroids, glycosides and anthraquinones. The antioxidant potential of flowers and seeds in the different solvents was evaluated. Their SC_{50} and EC_{50} values were determined to evaluate the therapeutic potential, in which seeds were found to have higher antioxidant activity revealed by lower SC_{50} and EC_{50} value. The total phenol, flavonoid, flavonol and tannin content were determined to study the free radical scavenging property. It was therefore concluded that the seeds were found to have higher antioxidant activity when compared to flowers in various solvent extracts indicating their pharmacological property.

Sathya *et al.*,[16] determined the phytochemistry of aqueous extract of *Cassia occidentalis*. The antimicrobial activity and antioxidant properties of the plant on *Escherichia coli*, *Pseudomonas* aeruginosa and *Staphylococcus aureus* was determined. Alkaloids, tannins, saponins, carbohydrate, glycoside, phytosterols, Oils and Fats, phenol, flavonoids, protein and amino acid were present in the leaves and seeds of the plant. It was concluded that the aqueous plant extract can be used as antimicrobial agent, antioxidant and also used as herbal medicine for curing number of disease in the form of pellets or paste.

MATERIALS AND METHODS

Plant collection and Identification

C*assia occidentalis* leaves were collected at the Botanical Gardens of Nigeria Natural Medicine Development Agency. It was identified and sample deposited with herbarium number MPNH/2017/1258 at Medicinal Plants of Nigeria Herbarium of NNMDA.

Preparation of plant extracts

The leaves were collected fresh and then air-dried under shade at room temperature for about 14 days. After drying, the plant materials were pounded 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.

Ethanol/Petroleum ether crude extract preparation

The pulverized plant samples (200 g) of *Cassia occidentalis* were soaked in 500 mL of the solvents. These mixtures were kept on the rotator shaker for 72 hours for accelerated agitated extraction. The mixtures were then filtered using Whatman filter paper grade 1. The filtrate were concentrated using water bath at 60^{o}C until the solvent was completely removed.

Hot water extract

The pulverized plant sample was placed in a blender and 1litre of hot distilled water was added to it. The mixture was then thoroughly blended for five minutes and then filtered using a Whatman filter paper grade 1. Excess water was removed using hot water bath and stored in airtight bottles until required.

Test organisms

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

Microbiological Assay

The microbial activity was determined by measuring the Zones of inhibition in mm using the method as described by Irobi *et al.*[17]

Formulation of creams

Oil in water cream was produced by adding the oil phase to the aqueous phase slowly after heating to between 70-75^{0}C with continuous stirring. Heating was continued at the same temperature for about 10 - 15 minutes. The coarse cream formed were then cooled to about 35^{o}C gradually. The cream was allowed to stay at room temperature for twelve hours and then homogenized with the aid of a mechanical stirrer. Three different containing 2% of *Cassia occidentalis* aqeous, ethanolic and Petroleum ether extracts were produced. The prepared herbal creams were then vigorously homogenized.

Physical evaluation of the formulations

The cream formulations were subjected to visual inspection for their colour, homogeneity, consistency and phase separation.

Measurement of pH

pH Meter Model 290 Mk 2 was used in the Laboratory of the Nigeria Natural Medicine Development Agency, Lagos in taking the measurement. The pH meter was calibrated with buffer solutions 4 and 7 before the sample solution pH was then taken.

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^{o}, 4^{o}, 30^{o}, 37^{o} C and 45^{o}. The formulated creams were subjected to Freeze thaw cycles testing which involves making the samples pass through three cycles of temperature at -10^{o} 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^{o} 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 is carried out on the cream to determine the sensitivity of the emulsions to the Ultra Violet radiation. This was carried out by placing the creams in test tubes and also in the actual package and then put in the window where direct sun rays fell on them.

Moisture loss on drying/ residue content at 105^{o}C

Cream (2.0 g) was weighed into a dried Petri dish and kept in the oven at 105^{o}C for 2 hours. It was taken and stored in a desiccator to cool. After cooling to room temperature, the sample was weighed. The procedure was repeated until the weight of the sample was constant.

RESULTS AND DISCUSSION

Percentage yield of extracts by the solvents are in the order petroleum ether (4.2%) < aqueous (4.6%) < ethanol (5.4%).

Antimicrobial Screening

Effect of aqueous extract of *Cassia occidentalis* against the microorganisms

As shown in Table 1, the activity of the extract at 10000 µg/mL was highest against *M. audounii* (11 mm) followed by *M. furfur* (9.4 mm). While it exhibited same activity against *T. Mentagrophtes* and *E. floccosum* (9.3 mm). At 1000 µg/mL, the activity was in the order *M. audounii* = *M. furfur* (7 mm) < *E. floccosum* (8 mm) < *T. mentagrophtes* (9 mm). No activity was recorded for the extract at 10 and 100 µg/mL respectively. Saganuwan *et al*.,[6] reported that the phytochemicals in the leaves of *cassia occidentalis* have definite pharmacological actions against some human pathogens. This view was supported by Yadav *et al*.,[1] who observed that when the activities of extracts of *cassia occidentalis* leaves were tested against selected bacteria and fungi, the aqueous extract was the most active and that it was more active against fungi than bacteria. During the experiment conducted by Bhagat *et al*.,[18] in determining the cyto-toxity of *cassia occidentalis* extracts against some human pathogens, it was concluded that the aqueous extract of the plant was more potent against alcoholic and hydro-alcoholic extract of the plant

Table 1: Zones of inhibition (mm) of aqueous extract on microorganisms.

Concentration in µg/mL	10	100	1000	10000
T. mentagrophtes	-	-	9±0	9.3±0.58
M. audounii	-	-	7±0.2	11.3±0.58
M. furfur	-	-	7±0.2	9.4±0.58
E. floccosum	-	-	8±0.2	9.3±0.58

Zone of inhibition in mm (mean± SD) of three replicates.

Effect of ethanol extract of *Cassia occidentalis* against the microorganisms

The activity of ethanol extract of the plant was highest against *E. floccosum* at 10±1 at 10000 µg/mL The activity was also noticeable against *T. mentagrophtes* (10 mm). However against *M. audounii* and *M. furfur*, the activity was the same. This observation can not be said to be the same for the extract at 1000 µg/mL. The activity is in the order *M. furfur* = *E. floccosum* < *M. audounii* < *T. mentagrophtes.* At 100µg/mL, activity was only noticed for *T. mentagrophtes* while no activity was observed against none of the dermatophytes at 10µg/mL (Table 2). Davariya and Vala,[20] in their work reported that the extracts performed good and almost better than the standard drugs Nystatin and Griseofulvin against some selected fungi with exception of against *aspergilli*. The activity of the extract can also be attributed to Mohammed *et al.*,[19] who reported that the extract exhibited varying activity against different microbes. They therefore concluded that the activities could be attributed to active metabolites present in the extract.

Table 2: Zones of inhibition (mm) of ethanol extract on microorganisms.

Concentration in µg/mL	10	100	1000	10000
T. mentagrophtes	-	8±0	8±0	10±0
M. audounii	-	-	7±0	9±1
M. furfur	-	-	6±0	9±1
E. floccosum	-	-	6±0	10±1

Zone of inhibition in mm (mean± SD) of three replicates.

Effect of petroleum ether extract of *Cassia occidentalis* against the microorganisms

At 10000µg/mL, the extract had activity against all the dermatophytes with *T. mentagrophtes* having the highest activity of (12±1mm), *M. audounii* and *E. floccosum* having the same activity (12±0 mm) and the least activity against *M. furfur* (10±0 mm). At 1000 µg/mL, the least activity was against *M. audounii* (7±0.1 mm) while the highest activity was against *E. floccosum* (9±0.1 mm). However at 10 and 100µg/mL, the extract was not active against any of the dermatophytes except *M. furfur* at 10 and 100µg/mL respectively. (Table 3). Though much work has not been done on petroleum ether extract of the plant but work carried out by Vedpriya *et al*.,(14) on the benzene and petroleum ether extracts of the leaves of *C. occidentalis* were reported to be effective against *P. mirabilis* and *E. coli* respectively while chloroform extract was found to be very inactive against all tested bacterial and fungal and yeast strains.

Table 3: Zones of inhibition (mm) of petroleum ether extract on microorganisms.

Concentration in µg/mL	10	100	1000	10000
T. mentagrophtes	-	-	8±0.2	12±1
M. audounii	-	-	7±0.1	12±0
M. furfur	5±0	7±1	8±0.1	10±0
E. floccosum	-	-	9±0.1	12±0

Zone of inhibition in mm (mean± SD) of three replicates.

Temperature Stability Testing

The results of changes in colour and pH from date of production through 2,4,8,12 and 16th week of production then stored at -10 and 4^{O}C are presented in table 1. It was observed that at production through 2,4,8.12 and 16th week, the samples remain stable as the storage temperature did not allow movement or dissociation of ions. It was also noted that there were no noticeable colour changes in the samples. At 30^{o}C, as presented in Table 4, the pH of the aqueous extract cream and the colour remain the same at production and after 2 weeks of production. However, the pH reduced to 7.15 from 4th to 16th week of production. This is probably due to the fact that there were no more dissociation of H ions in the cream. The pH of Co.Ethanol cream was 7.24 at production but by 4th week reduced to 7.22. At 8th week, it was 7.19 and the cream remained stable till 16th week of production. pH of 7.16 was measured for Co. petroleum ether cream at production and this reduced to 7.13 and remained stable at this pH all through 2nd week to 16th week of production. Results of the stability test carried out on the cream samples at 37^{o}C is presented in Table 5. There was sharp decrease in pH of all the samples but no colour change was noticed. Co. aqueous cream sample pH was 7.18 at production but by 16th week, it has reduced to 7.14 while that of Co. ethanol cream sample reduced from 7.24 to 7.17 at 2nd and 4th week and further reduced to 7.16 from 8th to 16th week of production. Same observation was made for Co. petroleum ether cream sample in which the pH was 7.16 at production but decreased sharply to 7.12 through 2nd week to 16th week after production. Table 6 shows the results obtained when the samples were stored at 45^{o}C. No change in colour was observed but sharp decrease in the pH was noticed for all the samples.

Table 4: Effect of (-10^{o}C) Temperature on cream samples after production.

Product	Day 1		2 weeks		4 weeks		8 weeks		12 weeks		16 weeks	
	pH	Colour	pH	Colour	PH	colour	pH	colour	pH	colour	pH	colour
Control	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC
Co..W	7.18	NCC	7.18	NCC	7.18	NCC	7.18	NCC	7.18	NCC	7.18	NCC
Co.E	7.24	NCC	7.24	NCC	7.24	NCC	7.24	NCC	7.24	NCC	7.24	NCC
Co.Pet	7.16	NCC	7.16	NCC	7.16	NCC	7.16	NCC	7.16	NCC	7.16	NCC

NCC - No change in colour Co.W – *Cassia occidentalis* water extract
CC - Change in colour Co.E – *Cassia occidentalis* Ethanol extract Co.Pet – *Cassia occidentalis* Petroleum ether extract

Table 5: Effect of (30^{o}C) Temperature on cream samples after production.

Product	Day 1		2 weeks		4 weeks		8 weeks		12 weeks		16 weeks	
	pH	Colour	pH	Colour	PH	colour	pH	colour	pH	colour	pH	colour
Control	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC
Co..W	7.18	NCC	7.18	NCC	7.15	NCC	7.15	NCC	7.15	NCC	7.15	NCC
Co.E	7.24	NCC	7.22	NCC	7.22	NCC	7.19	NCC	7.19	NCC	7.19	NCC
Co.Pet	7.16	NCC	7.13	NCC	7.13	NCC	7.13	NCC	7.13	NCC	7.13	NCC

NCC - No change in colour Co.W – *Cassia occidentalis* water extract
CC - Change in colour Co.E – *Cassia occidentalis* Ethanol extract
Co.Pet – *Cassia occidentalis* Petroleum ether extract

Table 6: Effect of (37°C) Temperature on cream samples after production.

Product	Day 1		2 weeks		4 weeks		8 weeks		12 weeks		16 weeks	
	pH	Colour	pH	Colour	PH	colour	pH	colour	pH	colour	pH	colour
Control	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC
Co..W	7.18	NCC	7.18	NCC	7.14	NCC	7.14	NCC	7.14	NCC	7.14	NCC
Co.E	7.24	NCC	7.17	NCC	7.17	NCC	7.16	NCC	7.16	NCC	7.16	NCC
Co.Pet	7.16	NCC	7.12	NCC	7.12	NCC	7.12	NCC	7.12	NCC	7.12	NCC

NCC - No change in colour
CC - Change in colour
Co.W – *Cassia occidentalis* water extract
Co.E – *Cassia occidentalis* Ethanol extract
Co.Pet – *Cassia occidentalis* Petroleum ether extract

Table 7: Effect of (45°C) Temperature on cream samples after production.

Product	Day 1		2 weeks		4 weeks		8 weeks		12 weeks		16 weeks	
	pH	Colour	pH	Colour	PH	colour	pH	colour	pH	colour	pH	colour
Control	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC
Co..W	7.18	NCC	7.14	NCC	7.14	NCC	7.14	NCC	7.14	NCC	7.14	NCC
Co.E	7.24	NCC	7.16	NCC	7.16	NCC	7.16	NCC	7.16	NCC	7.16	NCC
Co.Pet	7.16	NCC	7.12	NCC	7.12	NCC	7.12	NCC	7.12	NCC	7.12	NCC

NCC - No change in colour
CC - Change in colour
Co.W – *Cassia occidentalis* water extract
Co.E – *Cassia occidentalis* Ethanol extract
Co.Pet – *Cassia occidentalis* Petroleum ether extract

Light Testing

When the formulations were exposed to UV light, it was observed that they were not sensitive to the UV light. (Table 8)

Table 8: Light Testing.

Control	NCC
Co.W Cream	NCC
Co.E Cream	NCC
Co.Pet Cream	NCC

NCC =No colour change, CC= colour change

Centrifuge testing

No phase separation was detected in all the samples at 2000,2500 and 3000 rpm. However, at 4000rpm, phase separation was detected for Co.petroleum ether cream. This maybe due to the fact that the disperse phase was not well particulated in the continous phase which made the separation to occur or there is probability of the presence of a phytochemical causing the separation. Table 9.

Table 9: Centrifuge testing.

Sample	2000 rpm	2500 rpm	3000 rpm	4000 rpm
Control	NPS	NPS	NPS	NPS
Co.W Cream	NPS	NPS	NPS	NPS
Co.E Cream	NPS	NPS	NPS	NPS
Co.Pet Cream	NPS	NPS	NPS	PS

NPS - No phase Separation, PS – Phase Separation

Freeze-thaw testing

During cycle testing, there was no phase separation observed for both aqueous and ethanol cream samples (Table 10). However, phase separation was observed for petroleum ether cream sample. It is suspected that there is a presence of a phytochemical in the petroleum ether sample preventing complete particulation of disperse phase in the continuous phase.

Table 10: Freeze-thaw testing.

Control	NPS
Co.W Cream	NPS
Co.E Cream	NPS
Co.Pet Cream	PS

NPS - No phase Separation, PS – Phase Separation

Moisture loss on drying

As shown in Table 11, it was observed that *Cassia occidentalis* pet.ether cream moisture content was the

highest. This implies that the cream will be more susceptible to microbial attack compared to the other 2 cream samples.

Table 11: Determination of moisture loss on drying/residue content of antidermatophyte creams.

Cream	Moisture loss on drying (%)	Residue content (%)
Cassia occidentalis aqueous	6.39	93.61
Cassia occidentalis ethanol	6.36	93.64
Cassia occidentalis pet.ether	6.41	93.59

CONCLUSION

Overall, the present study indicates the dermatophytic properties of leave extracts of *C. occidentalis* and stability of the formulated creams for skin infection. This study paves the way for further attention and research to identify the solvent extract that can be used for stable production for dermatophytic infection and possibly determine the compounds responsible for the plant biological activity. Further studies would be undertaken to determine the efficacy of the herbal creams. It was observed that the most active solvent extract was petroleum ether extract, while all the formulations showed acceptable physical properties and were stable except for petroleum ether extract based cream formulation.

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.

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