Research Journal of Applied Sciences, Engineering and Technology 2(2): 133-137, 2010
ISSN: 2040-7467
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Submitted Date: September 04, 2009 Accepted Date: September 24, 2009 Published Date: March 10, 2010

Formulation and Antimicrobial Studies of Coconut (*Cocos nucifera* Linne) Oil

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Abstract: Coconut oil obtained from the nuts of *Cocos nucifera* was formulated into creams in order to standardize its use and present it in an elegant form. Using the fusion method, oil in water (o/w) creams were formulated in concentrations of 5 to 40% w/w of oil.The release of active ingredients from creams was investigated using cream challenge and skin inoculation tests, whereby creams were exposed to various spots on skin inoculated with *Ps. aeruginosa* ATCC 7853, *E. coli* ATCC 9637, *P. vulgaris* (clinical isolate), *B. subtilis* ATCC 607 and *C. albicans* ATCC 10231. In addition *A. niger* (clinical isolate) and *S. aureus* ATCC 13709 were used for antimicrobial screening.The stability of creams was also evaluated using a standard method. The results showed that active ingredients of the coconut oil were released from the creams; this was shown from the good antimicrobial activity of the cream confirming that all formulation ingredients were compatible and did not interfere with activity of the oil. The creams were also found to be stable, as a result of their ability to withstand shock and maintain their physical characteristics.

Key words: Antimicrobial, fusion, skin penetration

INTRODUCTION

The non-precise standardization of dosage and unhygienic practice environments in traditional medicine setups are factors militating against traditional medical practitioners. Such unethical practices could lead to preventable hazards such as therapeutic failure, toxicity, emergence of resistance and other adverse effects on the consumers. Formulation into designed dosage forms is a gateway towards scientific evaluation and standardization of crude drugs.

Skin infections are widely encountered in the tropics with lots of orthodox remedies involving the use of systemic antibiotics, the problems of drug resistance and reported allergies are also abound. Coconut oil has been confirmed to possess antimicrobial, antiviral and antiprotozoal activities (Isaacs and Thormar, 1991; Thormar, 1996; Enig, 2003). Phytochemical studies indicated that lauric acid which is its major fatty acid component was highly responsible for the activities of the oil (Peat, 2003). Lauric acid has been documented to be converted to monolaurin in the human body and it is the antimicrobial agent found in human milk (Peat, 2003). This study is aimed at carrying out formulation studies of the oil, with the view of utilizing compatible excipients and in compliance with cGMP. Standardization of oil quantity according to activity will also be carried out. Topical drug delivery has gained a lot of attention in the last 30 years. The most cosmetically acceptable emollient preparation is the cream due to its cosmetic appeal and the ease with which it is washed off the skin. The antimicrobial activities of coconut oil has been reported earlier by Obi *et al.* (2005), prompting the present attempt to explore its formulation into creams.

MATERIALS AND METHODS

This study was conducted in the Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria-Nigeria; November 2004 - February 2005.

Materials:

Coconut oil: This was obtained by extraction from coconut fruits sourced locally.

Micro-organisms: *Ps. aeruginosa* ATCC 7853, *E. coli* ATCC 9637, *A. niger* (clinical isolate), *S. aureus* ATCC 13709, *P. vulgaris* (clinical isolate), *B. subtilis* ATCC 607 and *C. albicans* ATCC 10231 which were all obtained from the Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria-Nigeria.

Media: Nutrient agar (Biotec), Nutrient broth (Lab.M), Sabouraud's dextrose broth (SDB) and Sabouraud's dextrose agar (SDA) were from Oxoid.

Methods:

Extraction of coconut oil: The fresh endosperm or coconut meat was separated manually from the shells and then washed to remove dirt. The product was cut into pieces (about 1.27 cm or 12.7 mm in diameter) using a

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knife. It was weighed and milled using a local crusher mill.
The resulting mass was mixed with lukewarm water and chaff was filtered out using a cotton cloth. (The ratio of milled meat to water was I:1). The residue (marc) was firmly pressed and process repeated to ensure thorough extraction of the milky liquid oil. This liquid was heated to boiling; at this point the source of heat was removed to avoid boiling over and wastage of milk.

Heating effected the floating of oil as the top layer. This was skimmed off and gradually evaporated to dryness over gentle heat in a shallow aluminum pan. Constant stirring to avoid burning and discoloration was carried out at this stage of drying. The oil was filtered off and the deposit or "brownie" was firmly pressed to release remaining oil. The oil was thereafter decanted. Finally, the product was gently heated to dry all traces of moisture and filtered through a cotton cloth to obtain the oil for further use.

Formulation of coconut oil creams: Oil in water creams (o/w) were selected for formulation due to the advantage of easy removal from skin. The concentration of coconut oil used for the formulation ranged from 5 – 40% w/w using the formula on Table 1. Caution was exercised to avoid contamination of cream during formulation by working in a clean environment.
The following procedure was generally adopted:

- The oil was weighed in a dry ceramic evaporating dish (200 ml capacity). Oil soluble ingredients such as cetostearyl alchohol, d α-tocopheryl acetate and the parabens were weighted separately and transferred to the oil. The mixture was melted over the water bath at a temperature of 70ºC. The temperature was not allowed to rise above 75ºC as parabens are decomposed around that temperature (Idson and Lazarus, 1986).
- Propylene glycol was weighted into a dry 100 ml beaker; water was measured out and added leaving behind about 10 ml. The sodium laurly sulphate and cetrimide were added, after which the mixture was heated to dissolve the ingredients and further raised to 75ºC.
  Note: Aqueous phase is generally required to be heated 5ºC above the oily phase, since it will be transferred to the later, otherwise, it will lower the temperature of the oily mixture and cause local congealing of the waxy components.
- The temperatures of the oily phase and the aqueous phase were taken using two different thermometers. The aqueous phase was then added gently and steadily to the oily phase without splashing and with continuous stirring using a big glass rod. At this temperature (70-72ºC), intimate mixing of the two phases occurred and the continuous phase was gradually added to the disperse phase. Initially w/o emulsion will be formed but as more water is added, phases will invert to a fine o/w emulsion (Idson and Lazarous, 1986).
- The hot mixture was then transferred to an insulating pad and stirring continued to ensure that the waxy components did not separate out. Cooling was slowed down to allow adequate mixing and prevent aeration.
- Perfume was added when mixture cooled to 43 – 45ºC to avoid chilling the cream and facilitate mixing of perfume oil in the incompletely congealed oil phase.
- The citric acid was weight and dissolved in the remaining water and solution gradually incorporated into the cooled cream.
- The pH of the cream was checked and adjusted to 4.5-6.5 where necessary using citric acid.
- Finally, the cream was cooled to 30-40ºC and passed through a hand homogenizer.
- For the batch containing bentonite and sodium lauryl sulpate, the preservatives were added to the oily phase together with d α-tocopheryl acetate. After melting, the bentonite was incorporated into the oily phase.

All creams were packaged into wide mouthed glass jars with plastic screw caps fitted with impermeable liners and properly labeled.

Tests on coconut oil creams:
Emulsion type determination;

Dye test: A drop of 1% amaranth solution was added to a small quantity of cream on slide, covered with a cover slip and examined microscopically under x40 magnification.

Sudan Red dye test: A few drop of 2% sudan red oily solution was incorporated into the cream on a slide, covered with a slip and examined microscopically.

Electrical conductivity test: The electrical conductivity of each of the creams using a conductivity Meter (Jerway, model 4010) was carried out as a confirmatory test for emulsion type.

pH determination: pH paper strips with colour indicators were used. Three readings were taken at once and recorded. An interval of 20-25 min was given for the paper to soak thoroughly after insertion into the creams.

Stability test: The accelerated stability test method by Garti *et al.* (1982) was used. According to this method subjection of cream to conductivity test before and after stress will indicate its stability. The magnitude of the conductivity differential between the two readings will indicate the degree of instability. The following procedure was adopted at room temperature:

- Cream was subjected to heat on water bath (60-62ºC) for ten minutes. It was removed and allowed to cool to room temperature. The process of heating and cooling was repeated.

- Conductivity measurements were repeated on all creams and results recorded.
- Steps (a) and (b) were repeated for two more days on a daily basis and results recorded.

Preservative efficacy test: The British Pharmacopoeia (1988) test for preservative efficacy of creams was adopted to test the efficacies of lemon grass oil, parabens and cetrimide.

Preparation of inoculum: The following organisms were used as test organisms

C. albicans ATCC 1023
A. niger (laboratory isolate)
S. aureus ATCC 13709
Ps. aeruginosa ATCC 7853

The bacteria were incubated for 18 to 24 h at 37ºC on nutrient agar slants while *Candida* and *Aspergillus* were incubated at 25ºC for 48 and 96 h, respectively on SDA slants. The cells were harvested using 10 mls sterile 0.1% peptone water containing 1% polysorbate 80. Serial dilutions were made from this stock to produce germ counts of approximately 10^8 sfu/ml. The bacteria cells were grown in nutrient broth and incubated at 37ºC for 18 h. The overnight cultures were diluted 1 in 10^3 for *S. aureus* and 1 in 10^4 for *Ps. aeruginosa* (Garrold *et al.*, 1963).

Antimicrobial activity testing on formulated creams: The following categories of creams were used for the test

- Cream preserved with methyl and propyl paraben
- Cream preserved with methyl, propyl paraben and cetrimide
- Cream preserved with lemon grass oil
- Unpreserved cream
- Each of the four tested creams was inoculated with 0.2 ml of the standardized culture
- The incorporation was done using sterile spatula to ensure even distribution. The cream was covered and stored at room temperature
- At the same time 0.2 ml of the incorporated suspension was added to 20 ml sterile 1% peptone water containing 1% polysorbate 80 (peptone water control)
- One gram quantity of the creams were removed at 0, 6, 24, 48 h; 7, 14 and 28 days. Each withdrawn sample was introduced aseptically into 9ml sterile 0.1% peptone water containing 1% polysorbate 80; two further step wise dilutions were made to produce 1:100 and 1:1000 dilutions using same broth.
- 1 ml aliquots of the last dilutions were each used to prepare pour plates with 20mls of nutrient agar or Sabouraud's dextrose agar as appropriate. These media were prepared with 0.5%w/v polysorbate 80 and 0.07% lecithin for the cetrimide containing cream. These served as neutralizers for the preservatives (Orth, 1979).
- Controls using one milliliter of diluted cultures on plain agar was done to confirm that the preservatives were not antagonistic to the micro-organisms.
- The peptone water controls were diluted and examined at the same time as the zero hour samples to obtain the initial number of micro-organisms per gram. This was used as the baseline for reduction in population of organisms with time.
- The population (cfu) of organisms recovered after 3 days incubation for bacteria and 5 days for fungi/yeast were determined. The difference in population at zero hour and after the incubation periods were recorded.

Test of activity of cream on skin:

- A 16 h culture of the organisms in above (preparation of inoculum) was standardized to contain 10^6 cfu/ml with sterile normal saline.
- The back portion of the hand, joints between the fingers was inoculated with the standard organisms above and dried
- Coconut oil cream was applied on the inoculated spots.
- Five minutes later the area was swabbed using sterile swabs
- The swabs were incubated overnight in 5 ml of sterile nutrient broth at 37ºC
- Tests were replicated and controls using normal saline in place of cream were carried out at the same time.

RESULTS

Emulsion type determination:

Dye test results: Microscopic examinations revealed that all creams were o/w. With amaranth; the emulsion background was pinkish with colorless globules. However with Sudan red which is oil soluble; globules were reddish against a colorless background.

Confirmation of emulsion type was done by the electrical conductivity test using the Jerway conductivity meter, where all creams conducted electricity and thus confirmed to be o/w.

Determination of pH: The pH values of all creams varied between 6 and 6.5 Batches (Table 1) A, B and E had pH of 6 while C and D were of pH 6.5; the recommended by BPC (1994) range is 4.5–6.5.

Conductivity tests: The results revealed that batches A and D had negligible variations while batches B and C showed higher variations (Table 2). Batch C separated into two layers after stress, while batch E was not tested because it was a colored product.

Table 1: Quantities of ingredients used for various batches

Ingredients	Quantities (g) and batches				
		A	B	C	DE
Coconut oil	40	45	40	50	40
Propylene glycol	4	4	4	4	4
Cetostearyl alcohol	7	5	2.7	2.7	-
Sodium lauryl sulphate	1	1	-	-	0.5
Methyl paraben	0.5	-	0.2	0.2	-
Propyl paraben	0.1	-	0.1	0.1	0.2
Citric acid	0.05	0.05	0.05	0.05	0.05
d- α-tocopheryl acetate		0.1	0.1	0.1	0.10.1
Perfume oil	0.4ml	-	0.4ml	0.4ml	-
Distilled water	46.85	44.34	42.15	42.15	49.65
Lemon grass oil	-	0.5ml	-	-	-
Cetrimide	-	-	0.3	0.3	-
Bentonite	-	-	-	-	5
Phenoxyethanol	-	-	-	-	0.5

Batches A and B are o/w creams containing anionic emulsifying agent, Batches C and D are o/w creams containing cationic emulsifying agent. Batch E contained anionic emulsifying agent and a finely divided solid.

Table 2: Conductivity measurement of creams before and after stress

S. No.	Batch	Conductivity (microsiemens)			
		Before stress	After; 1st day	2nd day	3rd day
1	A	138	127	139	145
2	B	141	156	193	210
3	C	100	87	59	Not taken, Separated
4	D	117	112	114	107

Batches A and D had negligible variation while B and C showed higher variation (Garti *et al* 1982)

Table 3: Effects of preserved and unpreserved creams on the survival of *A. niger* on exposure for different times

Cream type	0 h		6 h		24 h		48 h		7days	
	a	b	a	b	a	b	a	b	a	b
UNP	1	NG	NG	1	NG	NG	NG	1	From	
LGO	1	2	NG	5	NG		NG	NG	NG 7 days to	
PARA	1	NG	NG	NG	NG	NG	2	NG	28 days	
CET	4	1	1	NG	NG	1	NG	NG	ALL NG	
PWC	1	1								

UNP: Unpreserved cream, LGO: Cream preserved with lemon grass oil (batch B), PARA: Cream preserved with the Parabens (batch A), CET: Cream preserved with cetrimide (batch C), PWC: Peptone water control, a: 10^2cfu/ml, b: 10^3 cfu/ml, NG: No Growth

Table 4: Number of surviving colonies of *S.aureus* after exposure to creams

Cream type	0 h		6 h		24 h		48 h		7 days		14 days		28 days	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
UNP	NG	1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
LGO	NG	1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
PARA	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
CET	1	1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
PWC	TNTC	65												

a: 10^2cfu/ml, b: 10^3cfu/ml, TNTC: Too Numerous To Count

Table 5: Number of surviving organisms of *Candida albicans* after exposure to cream for different times

Cream type	0 h		6 h		24 h		48 h		7 days		14 days		28 days	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
UNP	NG	NG	NG	NG	NG	NG	NG	NG	1	NG	NG	NG	NG	NG
LGO	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
PARA	NG	NG	NG	NG	5	NG	NG	NG	NG	NG	NG	N	GNG	NG
CET	NG	NG	NG	1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
PWC	TNTC	400												

a: 10^2cfu/ml, b: 10^3cfu/ml, TNTC: Too Numerous To Count

Preservative efficacy test: The results indicated that all creams including the unpreserved one were protected and possessed antimicrobial activities (Table 3-6)

Skin activity test: All organisms did not survive the cream treatment as no viable organism was observed.

DISCUSSION

The formulated coconut oil creams exhibited both antibacterial and antifungal properties. This confirms that formulating the oil into cream does not affect its activity and that all excipients used did not affect the

Table 6: Survival of *Ps. aeruginosa* to cream on exposure for varying periods

Cream type	0 h		6 h		24 h		48 h		7 days		14 days		28 days	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
UNP	NG	3	NG	1	NG	1	NG	NG	NG	NG	NG	NG	NG	NG
LGO	1	NG	1	1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
PARA	1	2	1	12		2	NG	NG	NG	NG	NG	NG	NG	NG
CET	NG	3	1	NG	NG	1	NG	NG	NG	NG	NG	NG	NG	NG
PWC	TNTC	300												

a: 10^2cfu/ml, b: 10^3cfu/ml, TNTC: Too Numerous To Count

antimicrobial activity of oil. In contrast to the pure oil (Obi *et al.*, 2005), the cream formulations displayed a cidal activity. The active compound in the oil previously identified to be monolaurin could have had an enhanced penetration due to the presence of surface active emulsifying agents used in formulating the cream since emulsification of oils generally increases their absorptivity (Aulton, 1988). The choice of an anionic (sodium lauryl sulphate) and cationic (cetrimide) emulsifying agents were to avoid incompatibility with the selected preservatives notably phenolics and carboxylic acids (Pharmaceutical Codex, 1994). The antimicrobial activity of coconut oil had been attributed to the carboxylic acid – monolaurin metabolized to lauric acid in the body.

The preservative efficacy test indicated a synergistic action between the coconut oil and preservatives, which showed 100% kill at 0 h. This is in conformity with the finding of Thormar (1996) which reputed that monocaprin a component of coconut oil destroyed HIV, herpes virus and gonococci within a minute. Since the unpreserved cream showed similar results with the preserved, this indicates that preservation may not be necessary. The conductivity test carried out indicated that batches a and d were stable while b and c were unstable. The two stable batches had oil to water ratios close to 50:50 which has been generally observed with stable creams (Martins *et al.*, 1970).

CONCLUSION

This study has established that coconut oil can be formulated into an elegant cream which is active on both fungal and bacterial organisms. It also demonstrates the possibility of standardizing the quality and quantity of oil to be used therapeutically in extemporaneous preparations.

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