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Application and evaluation of *Cucumeropsis mannii* Naud. seed oil in methyl salicylate liniment and salicylic acid lotion formulations

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Abstract
Cucumeropsis mannii Naud. seed oil was obtained by soxhlet extraction using petroleum ether (40-60 °C). The oil was processed by bleaching, filtration, dewaxing, degumming and refining. The average values of the physico-chemical properties were determined. The oil was used to formulate batches of methyl salicylate liniment and salicylic acid lotion respectively. The products obtained were evaluated and compared with similar British Pharmaceutical Codex formulations of these drugs where castor oil was the oily component used. The liniment formulation containing *Cucumeropsis mannii* Naud. seed oil performed better than that containing castor oil; while the lotion formulations containing both oils had the same performance in terms of the amount of drug remaining at determined time intervals after storage.

Keywords: *Cucumeropsis mannii*, liniment, lotion, seed oil, methyl salicylate, salicylic acid

1. Introduction
Most of the fruits of the cucurbitaceae bear numerous seeds usually moderately rich in fat (20-35%), but up to now relatively little use of them has been made as a source of oil seed. Several investigators have previously suggested the possibility of exploiting this family as a commercial source of oil seeds [1, 2]. Many members of the cucurbitaceae or gourd family occur in Nigeria, and the seeds of several species and varieties are used locally as a source of cooking oil. Some of these oils have not yet been utilised on an industrial scale. *Cucumeropsis mannii* Naud. provides an important source of such seeds. *Cucumeropsis mannii* Naud. is a herbaceous climber reaching four meters height of forest areas and is indigenous to West Africa. It is commonly cultivated on farms and in gardens throughout its area of distribution [3]. The seeds, known locally as *agushi* (Hausa), *egusi-itoo* (Yoruba) or *ahu elu, aki* (Igbo) are removed from the flesh usually by stacking the fruit to allow decomposition to take place. The seeds are washed out after 10-15 days, and then dried and can be stored. They are prepared for consumption by parching and pounding to free the seed-coat from the kernel which can be eaten either raw or cooked, or more usually when ground to powder form it is added to stews. In flavour they resemble groundnuts. They are rich in oil and contain more protein than groundnuts. Amino acids are particularly well represented and are appreciably more abundant than in groundnuts. They are thus of high food value [3]. The oil is semi-drying. It is a good substitute for cotton-seed oil and is suitable for soap-manufacturing and for illuminating. It is used in cooking and can readily be refined into a superior product for table use [3, 4].
Lotion is a liquid preparation used externally for healing wounds, relieving pain and beautifying the skin. Salicylic acid which is employed in the preparation of lotions and solutions are used externally on the skin, where it exerts a slight antiseptic action and considerable keratolytic action. It is also combined commonly with zinc oxide, sulphur and coal tar. It is incorporated into mixtures for the treatment of acne, dandruff and seborrhoea, insect bites and stings and into soaps and vaginal douches, but efficacy remains to be established. In high concentrations it is caustic and may be used to remove corns, calluses, warts, and other growths. Salicylic acid is not employed internally as an analgesic because of its local irritating effect on the gastrointestinal tract [5-8].
Liniment is a liquid preparation rubbed on the skin, used to relieve muscular aches and pains. It contains some substances that when rubbed over the affected part causes mild irritation and often brings more blood to the painful part. Most liniments contain camphor, oil of turpentine or oil of wintergreen. Methyl salicylate is the main component of wintergreen. It is used in liniments to soothe muscular aches and is used medically to reduce fever, headaches and menstrual pain. Salicylates are useful, relatively safe, frequently used drugs, but large doses may cause hearing and vision difficulties and gastrointestinal disturbances [5-8].

2. Materials and Methods

2.1 Material

All chemicals used were of analytical grade. *Cucumeropsis mannii* Naud. seeds were purchased from a local market in Jos, Plateau State, Nigeria.

2.2 Physicochemical Properties

The physicochemical properties (specific gravity, density, relative density, refractive index, viscosity, oil yield, moisture content, ash content, organic matter content, iodine value, acid value, free fatty acid and saponification value) of *C. mannii* seed oil were determined using standard methods [9].

2.3 Extraction

The seeds were deshelled, screened, dried and ground using an electric blender. The oil was extracted in a Soxhlet apparatus with petroleum ether (40-60 °C) for 6 hours and solvent removed using a rotary evaporator.

2.4 Processing and Purification of the Extracted Oil

The extracted oil was bleached by treating with equivalent weight of activated charcoal and filtered with filter paper (dewaxed by boiling with n-hexane). The bleached oil was then degummed by warming to 35 °C with orthophosphoric acid, after which the acid was removed using a separating funnel. Then refining of the oil was achieved by warming the degummed oil to 35 °C (using a water bath) with 0.1N sodium hydroxide. The oil was then washed several times using distilled water and phenolphthalein indicator until no trace of sodium hydroxide was observed. Finally, the oil was placed in a hot air oven at 60 °C for about 15 minutes to allow separation and later decanted.

2.5 Formulation and Evaluation of Liniment

The formula for the methyl salicylate liniment consisted of 25% v/v methyl salicylate in *Cucumeropsis mannii* seed oil or castor oil. Methyl salicylate (12.5ml) was measured into a 50.0ml standard flask and sufficient processed *Cucumeropsis mannii* seed oil added to produce 50.0ml. After which the solution was properly mixed. The blank of the liniment was also prepared (without the methyl salicylate). The percentages of methyl salicylate remaining in the formulation after 1, 2, 3, 4, and 6 weeks were determined by withdrawing samples, adding 2 drops of ferric chloride, and reading the absorbance at 540nm in a colorimeter. The equivalent concentrations were then obtained from a standard Beer's plot. The above procedure was repeated using castor oil as a standard.

2.6 Formulation and Evaluation of Lotion

2.0g of salicylic acid was weighed into a 100ml standard flask and dissolved in a portion of ethanol (96%), after which the processed *Cucumeropsis mannii* seed-oil (1.0ml) and sufficient ethanol (96%) was added to produce 100ml and the solution properly mixed. The percentages of salicylic acid remaining in the salicylic acid preparation were determined at weekly interval for 6 weeks, by titrating 10.0ml of the lotion with 0.97N NaOH, using 2 drops of phenol red as indicator. The above procedure was repeated using castor oil as a standard. 10.0ml standard flasks were used to prepare blanks (lotion without the oils) of 12.50%, 25.0%, 50.0%, 75.0%, and 100% concentrations using 0.025g, 0.05g, 0.100g, 0.150g and 0.200g of salicylic acid respectively. The blanks were also titrated with 0.97N NaOH, using 2 drops of phenol red as indicator. Percentage concentration of the lotion at weekly interval was determined by extrapolation from the plot of the blanks against volume of base used for titration–calibration curve (table 1 and fig. 1).

Table 1: Titre values for the blank of the lotion

Concentration of Blank (%)	Volume of 0.97N NaOH used for 10ml of each blank (ml)
12.5	0.45
25.0	0.90
50.0	1.80
75.0	2.70
100.0	3.60

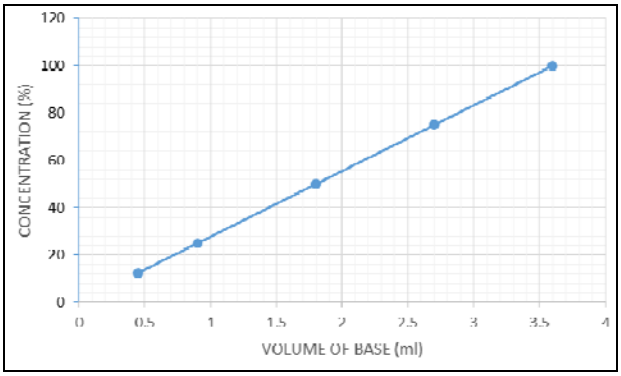

Fig 1: Graph of Percentage Concentration of Salicylic Acid of Blank Lotion vs the Volume of Base

3. Results and Discussion

3.1 Physicochemical Properties of *C. mannii* seed oil

The physicochemical characteristics of *C. mannii* seed oil are presented in table 2. The result shows that the percentage moisture content of *C. mannii* seed was found to be 3.058% which is a little lower than 5.9% reported by Mbuli-Lingundi *et al*. [10] This variation could be attributed to the prevailing climatic conditions (relative humidity in particular) in the areas concerned, the method of storage and the conditions surrounding it. Since the moisture content which is responsible for the enzymatic hydrolysis of oil under favourable condition to produce rancidity is low [11], it can be said that the seed oil of *C. mannii* is of very high quality. The organic matter and ash contents were found to be 95.917% and 4.083% (table 2) respectively. This indicates that the greater part of the seed contains combustible organic matters (e.g. carbonhydrates) and low concentration of inorganic materials (e.g. minerals). The percentage oil yield of the seed was found to be 48.74% (table 2); therefore, the seed of *C. mannii* can serve as a good source of industrially extractible oil. However, this value is a little higher than 40.37 and 45.0 earlier reported [3, 10]. The variation can be as a result of the soil and climatic conditions of the areas from which these seeds were harvested. The other physio-chemical properties shown in table 2, indicate that the oil is full of unsaturation, and is semi-drying. The extracted oil was found to be freely miscible with petroleum ether and n-hexane but absolutely immiscible with 96% ethanol and distilled water. The free fatty acid and acid values obtained were 0.675 and 1.35 respectively. The acid value is a measure of the extent to which the glycerides in the oil have been decomposed by lipase or other action, and rancidity is usually accompanied by free fatty acid formation [11]. Since the values obtained were relatively very low, the extent of rancidity is equally low; therefore, the oil can be used effectively for edible purpose.

The saponification value 140.79 is relatively high and shows that the oil can be useful in soap production.

Table 2: Physico-Chemical properties of *C. mannii* seed oil

Property	Value
Physical	
Specific gravity at 30 °C	0.3020
Density (g/mL)	0.9010
Relative density	0.9026
Refractive index at 30 °C	1.4731
Viscosity (Cp) at 25 °C	73.0000
Chemical	
Oil yield (%)	48.7400 ± 0.332
Moisture content (%)	3.0580 ± 0.152
Ash content (%)	4.0832 ± 0.013
Organic matter content (%)	95.9170 ± 0.013
Iodine value (Wij,s)	105.5000
Acid value (mgKOH/g)	1.3500
Free Fatty Acid (mgKOH/g)	0.6750
Saponification value (mgKOH/g)	140.7600

3.2 Analysis of the Prepared Liniments

The percentage of methyl salicylate remaining in the liniment preparations with castor and *Cucumeropsis mannii* seed oils upon storage at room temperature are represented in fig. 2 and on table 3. Fig. 2 showed that the concentration of methyl salicylate decreased to 80.65% after storage for one week with the castor oil formulation. The corresponding concentration in the *Cucumeropsis mannii* seed oil formulation was 95.16%. After three weeks of storage however, the value remained constant at 80.65% in the castor oil formulation, while it decreased slightly from 100% to 97.5% in the *Cucumeropsis mannii* seed oil formulation. Similar patterns of decomposition of methyl salicylate in castor oil and *Cucumeropsis mannii* seed oil preparations were 79.03% and 88.71% respectively. The relatively low rate of decomposition of the methyl salicylate in the formulations could be attributed to acid hydrolysis of methyl salicylate (an ester) in the presence of the fatty acids of the oil; since hydrolysis of esters is promoted not only by bases but also by acids [12]. For *Cucumeropsis mannii* seed oil, the free fatty acid and acid value (0.675 and 1.35 respectively) are very low. This also accounts for the low rate of methyl salicylate decomposition in the formulation, since the value of the free fatty acid which is supposed to catalyse the hydrolysis is very low. From these results, the amounts of methyl salicylate remaining in the *Cucumeropsis mannii* seed oil liniment after 1, 2, 3, and 4 weeks of storage at room temperature were all higher than the corresponding amounts in the castor oil liniment. Since the degree of stability of any preparation is to some extent, determined by the amount of the active ingredient remaining in that preparation after a given period of storage, it then follows that the preparation in *Cucumeropsis mannii* seed oil, which retained more of the active ingredient at all the time will be considered more stable than that in castor oil. This was further confirmed by the 90% value which was found to be less than one week and more than three weeks for the castor oil and *Cucumeropsis mannii* seed oil liniments respectively.

Table 3: Result of the Percentage Concentration of the liniments

Week	Castor oil liniment		*C. mannii* seed oil liniment	
	Absorbance	Concentration (%)	Absorbance	Concentration (%)
0	0.620	100.00	0.620	100.00
1	0.500	80.65	0590	95.16
2	0.500	80.65	0.610	98.39
3	0.500	80.65	0.605	97.58
4	0.490	79.03	0.550	88.71
5			0.550	88.71
6			0.540	87.26

The tabulated absorbances are the average values obtained, while the equivalent percentage concentrations were obtained from a standard Beer's plot

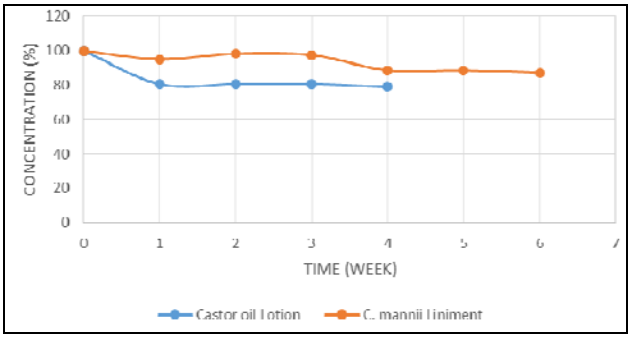

Fig 2: Percentage of Methyl Salicylate Acid Remaining in the Formulations

3.3 Analysis of the Prepared Lotions

The percentage of salicylic acid remaining in the lotion preparations with castor and *Cucumeropsis mannii* seed oils upon storage at room temperature are represented in fig. 3 and on table 4. Fig. 3 shows that the concentration of salicylic acid in the castor oil and *Cucumeropsis mannii* seed oil formulations remained constant from day one to the sixth week. This result shows that the degree of retention of salicylic acid in the lotion containing *Cucumeropsis mannii* seed oil is the same as in that containing castor oil which is usually the oil used in the formulation.

Table 4: Titre values for the lotion formulations

Week	Volume of 0.97N NaOH used for 10ml of Castor oil lotion (ml)	Volume of 0.97N NaOH used for 10ml of *C. mannii* seed oil lotion (ml)
0	3.6	3.6
1	3.6	3.6
2	3.6	3.6
3	3.6	3.6
4	3.6	3.6
5	3.6	3.6
6	3.6	3.6

The result shows average values

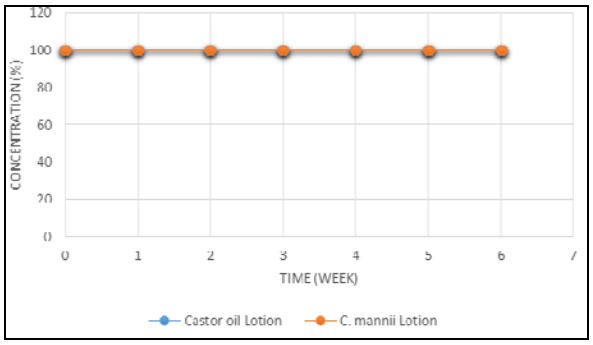

Fig 3: Percentage of Salicylic Acid Remaining in the Formulations

4. Conclusion

It has been shown in this work, that *Cucumeropsis mannii* Naud. seed oil which is a local, inexpensive and readily available oil in Nigeria, can comfortably substitute castor oil in the formulations of methyl salicylate liniment and salicylic acid lotion respectively. Also, considering the high percentage oil yield of *Cucumeropsis mannii* Naud. seed (48.74%), the plant can be included in the list of sources of oleochemicals used in the manufacture of cleaning agents, cosmetics, detergents, emulsifiers, insecticides, lubricants, paints, pesticides, pharmaceuticals and soaps. In addition to the above uses, the oil of *Cucumeropsis mannii* Naud. seed is of high quality and can be placed on the list of edible oils, since it has very low free fatty acids. Edible oils are consumed in various ways and are used in the manufacture of cooking fats and oils, salad dressings, ice cream and margarine.

5. Acknowledgement

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