COMPARATIVE EVALUATION OF MANGIFERA INDICA AND THEOBROMA CACAO SEED FATS AS SUPPOSITORY BASE.

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SUMMARY

Some properties of Mango seed fat useful in suppository base development for Nigeria climatic conditions have been investigated. The objective is to identify and charac e ize more locally sourced fatty bases of natural origin suitable for suppository dosage formulations. Dried mango seed fat content ranged between 5.8% and 8.4% depending on age of dried seeds and method of extraction. The melting point, displacement value and softening point of both test (mango seed fat) and reference (cocoa-butter) bases were similar. However, mango seed fat appeared superior to cocoa-butter with respect to brittleness of the finished product and dosage form stability, but showed slower drug release profile. More drug per unit weight of base was incorporated into cocoa-butter base compared to mango seed base. About 99% salicylic acid and 100% aminophylline were separately incorporated into 1g theobroma oil base whilst 87% salicylic acid and 98% aminophylline were similarly incorporated into 1g mango seed fat (250mg of each drug was used). Drug release from the test and reference bases was pH and storage temperature dependent with optimum release at pH 12.8 and 30°C. These results suggest that mango seed fat may be a good fatty base substitute for cocoa-butter in rectal suppository dosage formulations for use in the tropics.

Key words: Mango seed fat, fatty suppository base, physico-chemical properties, stability, in-vitro drug release.

INTRODUCTION

A variety of suppository bases have been used for the development of rectal drug formulations. Cocoa butter has been used as an official fatty base for over 200 years but it is characterized by a number of serious disadvantages including polymorphism, rancidity, adherence to mould and low softening point(1).

Synthetic triglycerides without some of these disadvantages have been developed but theobroma oil remains the only official natural fat with suitable properties for the preparation of suppositories [2,3]. Although most synthetic fats are superior to theobroma oil in many respects, the search for suitable suppository bases of natural origin is desirable, especially, in developing economies with abundant natural resources and low *per capita* income. The ideal suppository base should be stable to auto-oxidation, pour easily when melted, set quickly on cooling, easy to remove from the mould, and above all, should be compatible with most medicaments. Drug release should be good and predictable. Mango seed fat derived from ripe fruits of *Mangifera indica* Linn,, family Anacardiacea, was considered after some preliminary studies, to possess some of these properties.

In this study, the suitability of mango seed fat as a fatty suppository base of natural origin has been evaluated and compared with Cocao butter. Salicylic acid and aminophylline were the drugs of choice. Salicylic acid release characteristics from oleaginous bases are well documented in the literature [4-7]. Aminophylline suppositories are used in asthma chemotherapy especially for children. However, receiving adequate aminophylline release from suppository dosage form is a problem(8). This study compares drug release from rectal formulations of mango seed fat suppositories to official theobroma oil suppositories in order to determine the suitability of the test base for rectal dosage form development in Nigeria.

MATERIALS AND METHOD

MATERIALS

Cocoa butter, B.P. grade (BDH); mango seed fat was extracted locally from seed endosperm after the edible fleshy portion of the fruit has been removed, petroleum ether 40°-60°, sulphuric acid, and sodium hydroxide pellets were as supplied by B.D.H., salicylic acid (British Drug House), aminophylline (Macarthy Medical Ramford) reagents and all other materials were used as obtained from their respective suppliers without further purification.

EXTRACTION OF MANGO SEED OIL

The mango fruits used in this study were plucked very early in the morning (6am-7am) from selected trees growing in the University of Ibadan campus after they began to change colour, from green to yellow. On occasions they were picked when fully ripe fruits have just dropped from the tree. The fruits were identified and authenticated at the Botanical garden, Univesity of Ibadan. The fruits were washed, the stoney layer and edible pulp removed and the fresh seeds thoroughly washed in distilled water. The washed seeds were cut into small fragments and dried at 50°C, in a hot-air oven, to constant weight. The milled seeds were packed into extraction timbles and the oil content continuously extracted for 4h in a Soxhlet apparatus with petroleum ether 40°-60°. The expressed oil was centrifuged at 4000rpm for 30 minutes to separate foreign suspended particles from pure mango seed oil. The oil was left at room temperature to set to a semi-solid fat. The extraction process was done in several batches and the oil collected together in wide-mouthed amber coloured bottles which were sealed with aluminium foil.

Percent fat yield was determined to be in the range 5.75%w/w to 8.36%w/w.

PREPARATION OF SUPPOSITORIES

The displacement values of salicylic acid and aminophylline were determined for each base separately according to the official method [1,2]. The suppository bases in this study were:

(a) Freshly extracted mango seed fat (one week old);(b)Aged mango seed fat (one year old);(c) Cocoa butter B.P. (reference base);(d) Blend of mango seed fat and cocoa butter (1:1).

Suppositories were prepared by fusion using 1g steel moulds having six cavities. Test suppositories, containing 250mg drug, were prepared in batches of four sets i.e. 24 suppositories per batch. The moulded suppositories were allowed to cool to room temperature before storing at 4-8°C in a refrigerator for one hour. They were equilibrated again at room temperature for one hour before their removed from the moulds and final storage in screw-capped wide-mouthed bottles until required.

UNIFORMITY OF WEIGHT AND MIX

Twenty four suppositories were individually weighed and tested for uniformity of weight as described in B.P. 1980 [3]. Macroscopic and microscopic observations were carried out for uniformity of mix by slicing four randomly selected suppositories, from each batch, longitudinally into two equal halves for visual observation.

DETERMINATION OF DRUG CONTENT

From a set of six, the second, fourth and sixth medicated suppository were separately liquefied in 250ml purified water at 37°C with gentle stirring. One milliliter of the sample was diluted to 100mls with 0.1N sodium hydroxide solution or with distilled water after which drug content was assayed spectrophotometrically at 297nm and 270nm for salicylic acid and aminophylline respectively.

DISINTEGRATION TEST

Disintegration test of suppositories was determined using a specially designed equipment. The suppositories were placed under a wire gauze standing (tripod) 2cm from the bottom of 1 liter capacity beaker (pyrex) fitted with a 3-blade plastic stirrer (model Walgene 6160) held 3cm above the wire guaze. The beaker was filled with 600ml of distilled water and the suppositories were tested using constant temperature bath at 37±1°C for 60 minutes. The disintegration was observed for 60 minutes and an average of 6 determinations was recorded. The B.P. (1980) directs that disintegration for fat-based suppositories should occur in not more than 30 min (8).

DISSOLUTION TEST PROCEDURE

Drug release from test suppositories was studied by measuring dissolution rate of the

products using rotating basket method [9], with some modifications. Hanson's easi-lift six cylinder dissolution test station apparatus (Hanson's Research Laboratory) was employed. The influence of pH on drug release from the suppository formulations was studied using three media types:- (a) 0.1N sodium hydroxide (pH 12.8) (b) distilled water (pH 7.4) (c) 0.1N sulphuric acid (pH 2.8). At the start of each test, one unit of test suppository was placed in a dry basket which was lowered into the medium. The length of the drive shaft was adjusted to immerse the basket to a specific depth (USP XX 1990 [10].

Six hundred milliliters of media was used in place of 900mls recommended. The dissolution tester was rotated at 100rpm and the media maintained at 37+-1°C. At intervals of 10 minutes, 3ml samples were withdrawn from the dissolution medium and equal aliquots of pure medium added as replacement to maintain a sink condition. Each sample was filtered and assayed for drug content using UV spectrophotometry. The suppository product under test was prevented from floating to the top of the basket by means of a flexible wire mesh attached to the basket. The modifications applied in this test procedure did not affect the results significantly and helped to reduce cost.

RESULTS AND DISCUSSION

Table I shows fatty acid composition of locally sourced mango seed fat compared to cocoa butter (B.P. grade) as reference. The proportion of total unsaturated fatty acids (polyunsaturated) to total saturated fatty acids-P/S value in the base samples indicates that mango seed fat is more stable to auto-oxidation than cocoa butter. The P/S values for cocoa butter and shea-butter had been previously determined as 0.67 and 1.14 respectively [11, 12]. From Table I the P.S values for mango seed fat and cocoa butter are 0.07 and 0.66 respectively. The P/S value for cocoa butter hereby reported is over 9 fold that of mango seed fat which indicates that the latter is more stable to oxidation than the former. An important physical characteristic of vegetable fats found suitable for rectal suppository formulation is their melting point range. Table 2 shows the melting point ranges for both freshly extracted mango seed fat and aged mango seed fat as being almost identical to cocoa butter. The melting points were sharp, occurred over a narrow range and just below body temperature which suggests that both vegetable fats may be suitable in rectal suppository development. Lipophilic bases are chosen to melt just below body temperature which will aid the release and spreading of the medicament in the rectum and thereby promote drug absorption [12]. The softening point of the moulded suppositories was studied as part of the dissolution characteristics and the temperature at which the suppository products first changed shape and consistency was noted as the softening point. All samples tested softened between 32°-37°C. It has been reported that a fatty suppository base whose softening point was above 37°C took a very long time (*circa* 3 hours) to melt which could result in delayed drug release [13].

Table 1: Fatty Acid Composition of Mango Seed Fat and Cocoa Butter

Fatty Acid	Mango Seed Fat (wt%)	Cocoa Butter (wt%)
Total Saturated Fatty acids/glycerides	92.2 ± 1.8	60.8 ± 4.4
Total Unsaturated Glycerides	6.8 ± 0.4	40.2 ± 0.9

Values are means of determinations (n = 4) ± S.D.

Table 2: Melting Point Range of Mango Seed Fat and Cocoa Butter

Base Type	Melting Point (°C) ± S. D.
Freshly Extracted (one week old)	35.8 ± 0,4 to 37 ± 0
Aged mango seed fat (one year old)	35.4 ± 0.5 to 36.6 ± 0.5
Cocoa Butter (B.P. grade)	35.6 ± 0.5 to 36.8 ± 0.4

Visual observations of the moulded suppositories, when cut longitudinally, and microscopic examination carried out on sections of the samples revealed that the products were homogenous with minimal air entrapment. The true mould capacities, average weight of each set of suppositories were also determined and the data showed the suppositories met B.P. 1980 standards for uniformity of weight (4). Mango seed fat suppositories were found to be white, glossy, brittle, odourless with very attractive clean and polished appearance unlike the reference cocoa butter suppositories which were yellowish, sticky with chocolate-like odour. Thus, from the physical characteristics viewpoint, mango seed fat suppositories appeared superior to cocoa butter suppositories. Table 3 shows the displacement values of the two medicaments, salicylic.acid and aminophylline. Since the test and reference bases are natural fats, the displacement values are expected to be the same.

Table 3: Displacement values of Salicylic acid and Aminophylline for test and Reference Bases (replacement factor in parenthesis)

Drug	Mango Seed Fat	Blend mango seed fat/cocoa butter	Cocoa Butter
Salicylic acid	1.15 (0.88)	1.20 0.83)	1.26 (0.79)
Aminophylline	1.15 (0.88	1.22 (0.82)	1.31 (0.76)

However, the displacement values with reference to theobroma oil were higher for both medicaments than when mango seed fat was used in their formulation although the difference is not statistically significant ($p > 0.05$). The replacement factors are consequently lower for cocoa butter than mango seed fat which is due to the difference in their densities-mango seed fat being less dense than cocoa butter.

The contents of active drug in medicated suppositories shown in Table 4, reveal that about 87% and 99% of salicylic acid was incorporated into mango seed fat and cocoa butter suppositories respectively. Salicylic acid incorporation into fatty ointment bases and its release pattern had been fully documented. It is a drug of choice in comparative studies of drug release from oleaginous bases(4-7). The results of this study show that salicylic acid is well incorporated into fatty bases without significant stability problems. Aminophylline was incorporated to the extent of 98% and 100.00% in mango seed fat and cocoa-butter respectively. Aminophylline is a mixture of theophylline and ethylenediamine and its stability depends on pH(9). The salt is highly water soluble whilst the base component, theophylline, is sparingly water soluble. This might explain the higher entrapment efficiencies obtained with aminophylline compared to the more stable salicylic acid.

Table 4: Percent Drug Content in Prepared Suppositories

A: COCOA BUTTER				B: MANGO SEED FAT			C: BLEND MANGO SEED FAT/COCOA BUTTER (1 : 1)		
Drug (250mg)	Drug Content (mg)	Mg (%)	% Derivation	Drug Content (mg)	Mg (%)	% Derivation	Drug Content (mg)	Mg (%)	% Derivation
Salicylic acid	247.50	99.00	-1.00	217	86.80	-13.20	222.44	88.98	-11.02
Aminophylline	250.20	100.08	+0.08	245	98.00	-2.00	240.40	96.16	-3.84

The disintegration was taken to be complete when the moulded suppository had become soft with an appreciable change in shape and the mass floating under the wire gauze had no solid core when pressed with a glass rod. Disintegration test determines whether suppositories soften or melt within a prescribed time under prescribed experimental conditions. This gives an indication of the capacity of the suppository products to release their active medicaments after rectal administration. The release of insoluble medicaments from fatty bases is known to be directly related to the deformation time of the suppository(4). The BP 1980 recommends not more than thirty minutes for disintegration of fat-based suppositories and not more than sixty minutes for water-soluble suppositories(8). All the suppositories in this study disintegrated under thirty minutes thus indicating the potential for good bio-availability of incorporated medicaments.

Dissolution efficiencies of different suppository basal formulations enable ready comparisons to be made. Fig 1 shows the dissolution profiles of salicylic acid and aminophylline suppositories and the effects of base type and pH on drug release. Table 4 shows that each of salicylic acid and aminophylline suppositories contain approximately 250mg drug/gm base. the percent of drug released as a fraction of time depends on base type, pH of dissolution medium and nature of drug (fig. 1). Salicylic acid suppositories formulated with cocoa butter base exhibited faster dissolution rate compared to when it was incorporated in mango seed fat. Although drug release form mango seed fat was initially faster, dissolution slowed down after 20 minutes such that it could achieve only 86% drug release after 150 minutes as against 91% release from cocoa butter formulations. The effect of pH on dissolution rate, irrespective of drug type, is of the order pH 12.8 > pH 7.4 > pH 2.8. It should be noted that the percent of aminophylline released was consistently lower than percent of salicylic acid released throughout the experiment. Thus drug release was generally lower in acidic medium than in alkaline medium.

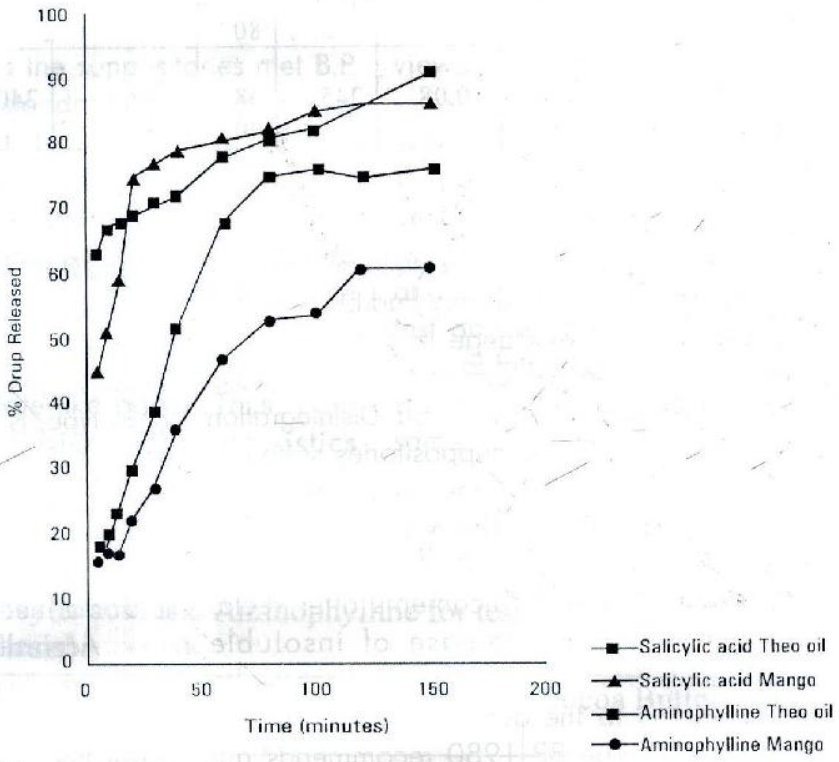
According to the pH-partition coefficient hypothesis, drugs with a high fat to water partition coefficient are liberated relatively slowly from fatty bases because aqueous solubility is important in maintaining adequate drug concentration gradient across lipid boundary and this phenomenon is pH dependent [14]. In this study seemingly contradictory result was obtained as salicylic acid release from fatty suppository base was found superior to aminophylline-a more polar drug. However, salicylic acid, because of its molecular size, diffuses rapidly through fatty bases but in aqueous medium, the pH determines the degree of ionization so that in acid medium salicylic acid would be mostly unionized whilst in alkaline medium, it would be mostly ionized. Hence salicylic acid was released to a greater extent in alkaline medium (pH 12.8) than in acidic medium (pH 2.8). Aminophylline is a salt of theophylline and ethylenediamine with higher molecular mass than salicylic acid. It is stable in highly alkaline pH but separates into component parts at low pH. The hydrophilicity of the drug would result in slow diffusion or permeation through fatty mass. Other factors known to influence drug release from rectal suppository products include viscosity of

melted suppository mass and mechanism of drug release-whether diffusion or partition limited [15].

CONCLUSION

This study has identified another natural fat, i.e. mango seed fat, as possessing good physico-chemical characteristics that recommend it for rectal suppository development suitable for use in tropical climate. Mango seed fat is more resistant to auto-oxidation, suffers no polymorphic changes and appears to possess better organoleptic properties than cocoa butter, the only official natural fatty base for extemporaneous preparation of suppository products. The drug release profile of this test base has been shown not to be as good as that of cocoa butter. However the modification of drug release characteristics by the use of certain additives such as surfactants could be considered in the formulation development of mango seed fat suppository products. An additional advantage of mango seed fat is the application of waste re-cycling technology in the processing since the base is derived essentially from waste materials. Thus the use of mango seeds for drug dosage form manufacture, if feasible, would be environment friendly.

Figure 1. Comparative dissolution profiles of Salicylic acid and Aminophylline suppository based formulations in 0:1 N NaOH medium (pH 12.8) at room temperature



Key:

Salicylic acid Theo oil – Salicylic acid in Theobroma oil base
Salicylic acid Mango – Salicylic acid in Mango-seed fat base
Aminophylline Theo oil – Aminophylline in Theobroma oil base
Aminophylline Mango – Aminophylline in Mango-seed fat base

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