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DEVELOPMENT AND *IN VITRO* EVALUATION OF ORAL GELS AND VAGINAL PESSARIES FROM CRUDE EXTRACTS OF FOUR GHANAIAN MEDICINAL PLANTS INTENDED FOR THE TREATMENT OF CANDIDIASIS

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ABSTRACT

Candidiasis is caused by the fungus candida and affects various parts of the body especially the vagina and the mouth. G-Rea® is a herbal mixture marketed in Ghana for the treatment of various candida infections. G-Rea® is prepared from various parts of four Ghanaian medicinal plants, namely: *Hoslundia opposita*, *Alstonia boonei*, *Combretum smeathmanii* and *Securidaca longepedunculata*. The current study was to compare the physicochemical and pharmaceutical characteristics of aqueous and ethanolic extracts of these plants and to develop them into vaginal pessaries and oral gels. Dried aqueous (A) and ethanolic (E) extracts were prepared by decoction and cold maceration respectively, and their physicochemical and antimicrobial properties were investigated. Extract (E) was formulated into oral gels and vaginal pessaries using glycerogelatine and theobroma oil as bases. The quality and antimicrobial properties of the formulations were evaluated against a number of microbes. A higher yield (17.3 %w/w) of ethanol extract was obtained compared to that of aqueous extract (9.51 %w/w). Both (A) and (E) were comparable in their organoleptic characteristics. Flavonoids were present in (E) in addition to other phyto-constituents present in (A). Both (A) and (E) showed activities against *S. aureus*, *B. subtillis* and *C. albicans*. Extract (E) generally showed wider zones of inhibition compared to (A) of same concentrations except against *S. aureus*. Oral gels (10 %w/w) and glycerogelatine vaginal pessaries of (E), were

stable, elegant and active against susceptible organisms. The study showed the successful formulation of the ethanolic extract of the four Ghanaian medicinal plants into oral gels and vaginal pessaries with demonstrable physicochemical quality and antimicrobial efficacy.

KEYWORDS: Candidiasis, herbal products, ethanolic extract, aqueous extract, oral gel, vaginal pessaries.

INTRODUCTION

Candida species are known human commensals that have the potential to cause opportunistic infections in man. Among the species, *Candida albicans* is considered to be one of the most virulent.[1] and is responsible for conditions such as vulvovaginal candidiasis, oral candidiasis, crohn's disease, ulcerative colitis and other serious systemic candida infections. Vulvovaginal candidiasis is an acute inflammation and is a common infection in matured women. Symptoms of this disease include copious vaginal discharge, pruritus, erythematous vulva and dyspareunia. A number of factors including diabetes mellitus, oral contraceptive usage and prolonged use of broad-spectrum antibiotics.[2] contribute to incidence of vulvovaginal candidiasis. The disease is also common in comorbid conditions.[3] and immune compromised individuals.[4-6]

Chemotherapy with antifungal agents such as miconazole, fluconazole, nystatin, amphotericin, voriconazole, and ketoconazole, among others, remains the first line treatment choice for candidiasis.[7] However most of these drugs have the potential to cause multiple congenital abnormalities when used during the first trimester of pregnancy. In addition, there are numerous side effects including electrolytes imbalance, nausea, abdominal abnormalities etc., aside being expensive. It is against this background that there is the need to continue the search for alternative treatment protocols, especially from plant sources.

The use of plants for medicinal purposes dates long before recorded history.[8] Natural products especially those of herbal origin have served as remedies for several human diseases such as diabetes, hypertension and parasitic infections including malaria and candidiasis.[9-14] In some cases natural products have provided lead compounds or yielded isolates for orthodox medicinal formulations. Mention can be made of quinine from Cinchona bark, reserpine from *Rauwolfia vomitoria*, artemisinin from *Artemisia annua*, just to mention a few. For most herbs, the specific ingredients that cause a therapeutic effect are not known. Whole herbs contain many constituents that are likely to work together to produce the desired

medicinal effect, such as synergy, enhancing mutual stability, and reduction of toxicity.[15] Isolating the active compound(s) may lead to attenuation of potency and reduced stability.

Herbal medicines are described as 'Plant derived materials or preparations with therapeutic or other human health benefits. They may contain either raw or processed ingredients from different parts of one or more plants.[16] Herbal medicine usage is common in many African and Asian countries because of the fact that they are readily available, relatively affordable and are believed to be safe.[17] A review of herbal medicine usage in 2008 suggests that up to 20 % of the population in the USA have used herbal remedies.[18] Other studies have shown that up to 20 % of the global population use herbal medicines resulting in about 2.5 billion dollar market for herbal products.[19, 20] Claims regarding safety are however debatable as herbs are not completely devoid of toxicity.[21, 22]

Herbal formulations come in different forms. These include decoctions, capsules, tablets, creams, gels, ointments, tinctures, suppositories and even some novel forms such as extended release, sustained release and microencapsulating dosage forms. [23] The most popular herbal preparations however are liquids derived from maceration, infusion and decoction. These herbal dosage forms usually have the disadvantages of having large dose volumes, packaging challenges and reduced stability. On the contrary, solid and semi-solid herbal preparations such as capsules tablets, ointment, powders and pessaries are more stable and are easier to standardize. Large scale production of herbal medicines due to commercialization requires manufacturers to maintain the quality and safety of these herbs and to ensure maintenance of their efficacy.

The herbal powder under study is a mixture of comminuted herbs used in the preparation of a liquid herbal mixture marketed in Ghana under the brand name G-Rea® herbal mixture. G-Rea® consists of various parts of *Hoslundia opposita* (family Lamiaceae), *Alstonia boonei* (family Apocynaceae), *Combretum smeathmanii* (family Combretaceae) and *Securidaca longepedunculata* (family Polygalaceae). The product is indicated for the treatment of *Candida albicans* infections specifically, oral thrush and vulvovaginal candidiasis. The component herbs in G-Rea® are reported by several researchers to have several pharmacologic and microbial activities [24-27] and are used to treat various ailments traditionally. Considering the range of diseases that can be treated with each of these herbs, it is clear that preparations containing the right amounts of these herbs may lead to synergistic effect, enhance efficacy and reduce toxicity compared to the individual components. The

current available formulation (G-Rea® herbal mixture) is a liquid dosage form with potential stability problems requiring the addition of preservatives. The large dose (60 ml) of G-Rea® mean patients may be taking high levels of such preservative.

The goal of this current study was to formulate oral gels and vaginal pessaries containing extracts of these herbal products that would be more convenient than the available liquid mixture. It is hoped that findings from this study would increase the scope of use of these herbs and also improve product stability and patient acceptability. It will also ensure reproducibility of product quality, and accurate dosing.

MATERIALS AND METHODS

Materials

Raw G-Rea® herbal powder mixture (sieve # 20) and G-Rea® herbal mixture (decoction) were obtained from Osei Herbal Clinic, Kumasi, Ghana. Theobroma oil was a gift from West African Mills Company (WAMCO), Takoradi, Ghana. Carboxymethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC) and aspartame were obtained from UK Chemicals, Kumasi, Ghana. Xanthan gum, potato starch, arachis oil, lactose, beeswax, glycerol, gelatine powder, ethanol 96%, benzoic acid, concentrated hydrochloric acid and MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, were obtained from the chemical stores of the Departments of Pharmaceutics and Pharmaceutical Chemistry, KNUST. Distilled water was always freshly prepared. Test organisms were *E.coli* ATCC25922, *Pseudomonas aeruginosa* ATCC 4853, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* NTCC10073 and *Candida albicans* (clinical strain).

Preparation of decoction and dried crude extracts

Crude extracts were prepared based on the formula and method (unpublished) of the producers of G-Rea® herbal mixture with modifications. Briefly, the powder mixture (50 g) was slowly boiled with 600 ml of purified water for one hour and allowed to cool to 40 ^{o}C. This preparation was then made up to 660 ml with purified water and labelled as 'Product A'. Ethanolic dry extract was prepared by a process of cold repeat maceration**.** A 100 g of raw powder mixture commonly used for the preparation of G-Rea® herbal mixture was cold macerated with 1 L of 70 % ethanol over 72 hours with occasional agitation. The preparation was filtered and the residue was further extracted with 1 L of 70 % ethanol for another 48 hours. Triplicate amounts of ethanolic extracts (60 ml, equivalent to a dose of G-Rea®) were transferred into previously weighed petri dishes and heated at 60 ^{o}C in a hot air oven to

dryness. The petri dishes containing the dried samples were weighed to determine the quantity of dried extract per unit dose (60 ml). This procedure was applied to the aqueous extract. The results were also used to compute the percentage yield based on the formula below:

Yield % = (weight of dried extract obtained / weight of the raw herbal powder mixture) x 100

Phytochemical screening of the dried extracts and decoctions

Phytochemical screening.[28] was carried out on the crude powdered samples, herbal mixture and the extracts to establish the major phyto-constituents present in them.

Solubility testing in water and ethanol

This was done using a prescribed WHO procedure.[29] for quality control of herbal materials with slight modifications. A quantity of each extract (1 g) was weighed into separate 50ml glass-stoppered conical flasks each containing 25 ml of respective solvents and the stopper inserted. Each was macerated for 24 hours, shaking frequently during the first 6hours. These were filtered and 10ml of each filtrate was dried at 105 ^{o}C to dryness, and then to constant weights in tarred petri dishes. The petri dishes and contents were quickly transferred into a desiccator and left for 30 mins to cool and weighed immediately. The amount of material in each petri dish was taken to be the quantity dissolved in the respective solvent.

pH and organoleptic properties

The pH of the decoction was determined using a pH meter (Oaklon Instruments) equipped with a glass electrode. Organoleptic characteristics including taste, colour odour and clarity of the extracts were determined.

Moisture content determination

Two grams of each of the extracts were placed in porcelain crucibles (which had previously been dried to constant weights) and weighed. The contents were then dried at 105 °C for 3 hours in a hot air oven and again weighed after cooling. Drying and weighing was continued at one hour intervals until constant weights were obtained. Triplicate determinations were carried out on each sample. The moisture content or loss on drying was expressed as a percentage of the extract.[29, 30]

Bulk and tapped density determination

Thirty grams of the powdered ethanolic extract was weighed and gently transferred into a 100 ml measuring cylinder and the initial volume (Vo) was recorded. The cylinder was then mechanically tapped 100 times to obtain a final volume Vf. The fluff density, consolidated density, Hausner ratio and the percentage compressibility were calculated using the equations below:

The fluff density $(D_o) = \frac{mass}{Vo}$.

The consolidated density $(D_f) = \frac{mass}{Vf}$

Hausner's ratio $= \frac{Df}{Do}$

Percentage compressibility (Carr's index) = $\frac{D_f - D_o}{D_f}$. [31]

Determination of angle of repose

The angle of repose (θ) of the ethanolic extract was determined by the fixed height method. [32] A funnel was clamped with its tip 10 cm above a paper placed on a flat horizontal surface. The powdered extract was carefully poured through the funnel to form a cone. The radius (r) and height (h) of the cone were used to calculate the angles of repose using the relation: tan θ = h/r.

Antimicrobial activity of dried extracts and decoction using Agar Diffusion method

The test bacteria used were *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtillis* and the fungi used was *Candida albicans.* Stabilized nutrient agar (20 tubes of 20 ml each) were seeded with 0.1 ml of 24 hour broth culture of the test organisms and each rolled in the palms to allow for thorough mixing of the organisms. Each tube was then poured aseptically into sterilized petri dishes and allowed to set at room temperature. Wells were bored using a sterile cork borer size 5 with internal diameter of 10 mm and labelled appropriately. Each well was filled with one of the different concentrations (1.25, 2.5, 5 and 10 %w/v) of the ethanolic and aqueous dried extracts in water. The plates were allowed to stand for one hour on the bench to enable diffusion of the extracts into the agar at room temperature. The plates were incubated overnight (24 hours) at 37 °C and the zones of inhibition were measured and recorded.

The above procedure was used in the antifungal activity determination using *Candida albicans* in Sabouraud agar. The antimicrobial activity tests were repeated for G-Rea® herbal

mixture sourced from the market. In this determination, the wells were filled with the decoction. All tests were done in triplicates.

Determination of minimum inhibitory concentration (MIC) of extracts using broth dilution method

This study was undertaken based on a procedure employed in previous studies.[33, 34] Calculated volumes of the extracts in test tubes were serially diluted with double strength nutrient broth and 0.1 ml of 24 hour broth culture of the test organisms were added to each tube. The required volume of sterile water was then added to make it up to 2 ml, to obtain the needed concentrations of 8, 7, 6, 5, 4, 3, 2, and 1 %w/v. A ninth and tenth test tubes were used as growth and sterility controls respectively. The sterility control tube was not inoculated with the test organism, while the growth control tube was inoculated with the test organism but without the drug. The tubes were covered and allowed to stand on the bench for about 30 minutes before incubating at 37 °C for 24 hours. To determine bacterial growth, 1ml of 125 mg/ml MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was added to each of the tubes and incubated for 10 minutes. Tubes with bacterial growth changed colour from yellow to blue while those with no bacterial growth retained the colour of the MTT. Tubes with the minimum concentrations that inhibited the growth of the test organisms were recorded as the minimum inhibitory concentration.

Formulation of oral gels

Two concentrations of the herbal extract (8 and 10 %w/v) were selected based on the MIC of the extract in order to obtain a product with effective antimicrobial activity. Different polymers were tested as gelling agents for the extract in order to obtain a stable product. The gelling agents used are as indicated in the formulae below (Table 1). There were eight formulae each corresponding to formulations labelled I – VIII. The concentrations of the gelling agents were selected in order to get a product whose viscosity was similar to that of Daktarin® oral gel (the reference product).

Table 1: Formula for preparation of oral gels

Ingredients	Quantities
Extract	8.0 g
Water/ethanol mixture (1:1)	6.0 ml
Glycerol	4 ml
Aspartame	0.2 g
Cocoa flavour	0.2 ml

Orange flavour	0.2 ml
Carboxymethyl cellulose (2%w/v)	to 100.0 g

The same formula was used for the other seven formulations except that the concentration of the extract was raised to 10 %w/w (10 g) and the gelling agents used were 4 and 6 %w/v CMC for formulations II and III respectively, 10 %w/v pre gelatinised potato starch for IV, 3 % w/v xanthan gum for V and 20 %w/v hydroxypropyl methylcellulose (HPMC) for VI, VII and VIII. Orange and white emulsion colours were added to formulations VII and VIII respectively to improve appearance. Other ingredients used were glycerol as a preservative, aspartame as sweetener, orange and cocoa flavours, ethanol and water for dissolution of extract. Orange colour and white emulsion colour were added to two of the formulations with 20 %w/v HPMC as the gelling agent to test for the effect of colour on the products.

In the formulation of gels I-VI, the quantity of each ingredient to be used was calculated as in Table 1. Weighed quantities of the powdered extract were triturated in the hydroalcohol (1:1) and mixed with aspartame and glycerol in pre-weighed mortars. These were well triturated to form smooth pastes. Enough of the prepared gelling agents were added and well mixed to obtain a smooth gel and to the required weight of 100 g. In the formulation of gels VII and VIII, orange and cocoa flavours were added before the addition of the gelling agent. All formulations were prepared at room temperature (30 ^{o}C). The physical characteristics of the gels were observed.

Antimicrobial activity of oral gels

The antimicrobial activities of the various gels prepared were determined using the agar diffusion method as described for the extracts. Here, the various gels were introduced into wells created in nutrient agar. The activity of the products against *Candida albicans* were compared to that of Daktarin® oral gel. The antifungal efficacy study against *Candida albicans* was determined by agar diffusion method employing cup plate technique.[35]

Determination of pH and viscosity of oral gels

The pH of the oral gels was determined as described earlier for the extracts except that dissolved gels were left for only 2 hours prior to measurements. Viscosities of the gels were carried out on a cone and plate geometry viscometer (Brookfield, Massachusetts, USA), using spindle No 40, at 250 rotations per minute at room temperature (30 ^{o}C). Evaluations were done in triplicates and mean viscosities were calculated. [35] The viscosity and pH of the

gel formulations were also tested weekly for 8 weeks as a means of evaluating the influence of storage time on viscosity and pH.

Preparation of vaginal pessaries

Bases used in this formulation were theobroma oil+10 %w/w beeswax and glycerogelatine. The 2 g pessary moulds were cleaned and lubricated with liquid paraffin and soap-spirit for glycerogelatine base and theobroma oil base respectively. Twenty grams of each base was weighed, melted and poured into the moulds. The best five of the resulting pessaries were selected, weighed and recorded as weight of blank pessary. In another formulation, 30 %w/w of extract was incorporated into the bases and five each of the resultant pessaries were also weighed and recorded as weight of medicated pessary. The displacement values were calculated using the equation below:

$$\text{Displacement value} = \frac{\text{weight of medicament}}{\text{weight of base displaced}}$$

Formulation of pessaries with extract was based on the weight of dried extract in one oral dose of decoction already obtained as 430 mg. The displacement values were found to be 0.51 and 1.25 for glycerogelatine and theobroma oil+beeswax bases respectively. Batches of 70 pessaries were prepared. The formula for each preparation is shown in Table 2 below.

Table 2: Formula for preparation of pessaries

Ingredients	**Quantities**	
	Formulation a	Formulation b
Dried powdered extract	30.10g	30.10 g
Theobroma oil +beeswax	115.9 g	-
Glycero-gelatine	-	81.0 g

Quality evaluation of pessaries

The pessaries were evaluated for physical appearance, shape and colour (observed visually), and texture or surface conditions. Samples were cut open to observe the presence or otherwise of bubbles. The uniformity of weight and disintegration time of the pessaries were determined using the approach of Saleem et al.[36]

Determination of antimicrobial activity of formulated pessaries

A pessary was slowly dissolved in about 5 ml of freshly distilled warm water. This was followed by the procedure previously used to determine the antifungal activity of the dried extracts and the decoction with minor modifications. In this method, wells were filled with

the pessary solution using Sabourauds agar and *Candida albicans* as the medium and test organism, respectively.

RESULTS AND DISCUSSION

Extraction yield

Aqueous and ethanolic extraction of the herbal mixture yielded 9.51 and 17.30 %w/w respectively. The higher yield obtained with ethanol could be attributed to a higher solubility of the components in 70 % ethanol than in water. Though water is considered to be the preferred solvent for pharmaceutical preparations due to its safety and chemical inertness, aqueous extracts are prone to microbial contamination compared to alcoholic extract especially if the material is macerated over a long period. The higher extractive value of ethanol in this extraction process thus makes it the preferred solvent.

Phytochemical and antimicrobial tests

The phytochemical screening of the crude powdered samples, herbal mixtures and the extracts revealed the presence of various phytochemicals which included; alkaloids, tannins, flavonoids, terpenoids, glycosides and saponins. Steroid, was absent in all the samples but saponins were present in all, an indication that these were triterpenoidal saponins. [37] The raw powder and the ethanol extract contained flavonoids whiles the aqueous extract and the herbal mixture sold on the market and Product A did not, even though, flavonoids are generally water soluble secondary metabolites. The absence of flavonoids in the aqueous extract may be due to the boiling procedure used. The phytochemicals detected in the extracts are reported to have various pharmacologic and antimicrobial properties.[38] It was therefore clear that the antimicrobial activity of the extracts may be attributed to the presence of some of these phytochemicals.

The antimicrobial study on aqueous dispersions of different concentrations of both extracts and the decoction (G-Rea® herbal mixture) revealed activities against *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* and non-activity against *Escherichia coli* and *Pseudomonas aeruginosa*. The zones of inhibitions of these susceptible organisms indicated contrasting results with ethanol extract generally showing wider zones than aqueous extract at comparable concentrations except *Staphylococcus aureus*. The extent of growth inhibition of these susceptible organisms was concentration dependent for both extracts (Table 3).

Table 3: Zones of inhibition (mm) of extracts and the decoction (n=3)

Organism	Ethanol extract				Aqueous extract				Decoction
	1.25 %w/v	2.50 %w/v	5.00 %w/v	10.00%w/v	1.25 %w/v	2.50 %w/v	5.00 %w/v	10.00%w/v	
S. aureus	11.33±0.58	16.33±0.58	19.33±0.58	30.67±1.10	0	16.33±0.58	21.67±0.58	26.67±0.58	12.66±0.58
B. subtilis	0	11.33±0.58	13.67±0.58	17.33±0.58	0	0	11.33±0.58	17.33±0.58	11.67±0.58
C. albicans	0	0	13.00±1.00	15.67±0.58	0	0	0	12.67±0.58	13.66±0.58

Diameter of cork borer =10 mm

Table 4: Minimum inhibitory concentrations (MIC) of the extracts against susceptible organisms.

Organism	MIC (mg/ml)	
	Aqueous extract	Ethanolic extract
S. aureus	20	20
B. subtilis	40	40
C. albicans	60	50

From Table 4 above, the minimum concentration of both extracts required to inhibit *Staphylococcus aureus* and *Bacillus subtilis* was the same, however, the ethanolic extract gave a lower MIC for *Candida albicans* indicating higher susceptibility of this fungus to the ethanolic extract. Since the herbal mixture is sold as antifungal, formulation of other dosage forms should target the use of the ethanolic extract, rather than the aqueous extract.

Table: 5: Physicochemical properties of extracts and herbal mixture.

Property	Ethanol extract	Aqueous extract	G-Rea® herbal mixture
pH	4.95	4.54	3.68
Solubility (g/ml) in 70%v/v ethanol	0.0042	0.00289	N/A
Solubility(g/ml) in water	0.003	0.004	N/A
Moisture content (%v/w)	9.07±0.12	10.23±0.25	N/A
Taste	Bitter	Bitter	Bitter
Colour	Greenish brown	Greenish brown	Yellowish brown
Texture	Powdery	Sticky and lumped together	N/A
Clarity	N/A	N/A	Clear

N/A = not applicable

Organoleptic properties considered are as indicated in Table 5. The ethanolic extract was free flowing powder and that of the aqueous extract formed semisolid plastic masses (pilular). Both extracts were greenish brown and the herbal mixture yellowish brown in colour, an indication of probable presence of leaves, stem bark and/or roots (bark) in the crude powder mixture. The extracts and herbal mixture were bitter even though the mixture contained aspartame as a sweetener. The herbal mixture was a homogeneous solution with no particles or deposits.

From the solubility results (Table 5), about 238 and 333 ml of 70 % ethanol and distilled water respectively were required to dissolve 1g of ethanolic extract and 1g of aqueous extract dissolved in 346 and 250 ml of 70 % ethanol and distilled water respectively. From BP 2013, these extracts were slightly soluble in these solvents. Poor water soluble drugs often require high doses in order to reach therapeutic plasma concentrations after oral administration. Considering the targeted dosage forms, the aqueous solubility of the ethanolic extract was good for the achievement of the desired therapeutic response.

The pH of the herbal decoction was 3.68 and that of the ethanolic and aqueous extracts were 4.95 and 4.54 respectively (Table 5). The low pH of the herbal mixture could be due to the presence of benzoic acid used as preservative. The pH values of the extracts (Table 5) were suitable for use in the preparation of vaginal pessaries and oral gels. The normal vaginal pH for a healthy vaginal function is between 3.8 and 4.5. [39] Formulating pessaries with extracts whose pH is within the normal pH range of the vagina can lead to products which will achieve its therapeutic purpose without disturbing the normal microbial flora of the vagina. Also the drug's pH would not be altered to affect its stability and efficacy.

The moisture content of the ethanolic extract (Table 5) was 9.07±0.12 %v/w and that of the aqueous extract was 10.23±0.25 %v/w. these values are within the generally accepted limit of 10 %v/w. Moisture content is an important quality control parameter for herbal extracts as it gives an indication of the stability of extracts on storage because high moisture content of an extract could lead to microbial contamination and/or chemical instability on storage. [29] Lower moisture content is always desired in powders. Though the values obtained are within limits, they are reasonably high to warrant protection of the extract from environmental moisture by storing in tightly closed containers or in desiccators.

Considering the antimicrobial and physicochemical properties of the extracts it was clear that the ethanolic extract was the extract of choice for formulation of other dosage forms other than the liquid herbal mixture. The in-house formulations in this study were oral gel and vaginal pessaries.

Flow properties of ethanolic extract

Knowledge of the flow properties of powders is important as it affects operations such as mixing, tablet compression, and capsule filling. The ethanol extract had good flow properties (Table 6) as all the flow parameters fell within the acceptable range.[40] of scale of flowability.

Table 6: Flow properties of ethanol extract of herbal mixture

Property	Mean value	*Standard range
Bulk density (g/ml)	0.54±0.01	N/A
Tapped density (g/ml)	0.62±0.01	N/A
Hausner ratio	1.15±0.02	1.12-1.18
Carr's index	13.25±1.71	11-15
Angle of repose	34±1^{o}	31-35

**For good powder flowability; N/A = not applicable; Mass of extract: 30 g*

Physicochemical properties of the oral gels

Organoleptic characteristics of pharmaceutical formulations especially oral dosage forms are important as they influence patients acceptability and hence compliance. Organoleptic profiling of the oral gels showed dark brown formulations, except batches VII and VIII (both containing 20 %w/v HPMC as gelling agent) which were yellowish brown and light brown respectively. These two batches (VII and VIII) also contained orange colour and white emulsion colour respectively which contributed to their different colouration. All the gels were translucent in appearance and showed good homogeneity and excellent consistency. All were sweet with bitter after taste except gel VIII, which was sweet with sharp after taste. The sweet taste could be due to the addition of sweeteners (i.e. aspartame and glycerol) which masked the bitter taste of the extract, while the bitter after taste was due to the strong bitter taste of the extract. All gels had pleasant orange/chocolate odour due to the addition of orange and cocoa flavours. Batch VIII appeared to be most elegant in terms of colour due to the white emulsion colour in it. All preparations were easy to wash on touch and did not stain the tongue after taste, except product VII, which stained both fingers and tongue. The staining property of batch VII was due to the presence of the orange colour.

Table 7: pH and viscosities of oral gels (n=3)

Formulation	pH	Viscosity (cp)
I	5.1±0.00	68.00±0.0
II	5.2±0.01	70.50±0.5
III	5.53±0.01	80.00±0.0
IV	4.78±0.01	71.00±0.0
V	4.67±0.01	69.67±0.58
VI	5.25±0.0	67.00±0.0
VII	5.19±0.01	66.87±0.50
VIII	5.12±0.00	66.33±0.58
Daktarin® oral gel	5.17±0.01	67.67±0.58

From Table 7, it was obvious that the pH and viscosity of most of the batches of oral gels were similar to the reference drug, Daktarin® oral gel.

Antimicrobial activity of oral gels

In vitro extract release from gels was evaluated using the antimicrobial properties of the extract at concentrations of 8 and 10 % w/v used to prepare the gels. Gel I (with 2 % CMC gelling agent and 8 %w/w of extract) and gel III (with 6% CMC gelling agent and 10 %w/w of extract) showed no noticeable antimicrobial activity against susceptible organisms (Table 8).

Table 8: Antimicrobial activity of oral gels (n=3)

Formulation	Mean zones of inhibitions (mm) + SEM		
	C. albicans	***B. subtilis***	***S. aureus***
I	0.00	0.00	0.00
II	12±0.00	0.00	14±0.56
III	0.00	0.00	0.00
IV	13±0.00	0.00	16±0.56
V	18±0.56	0.00	22±0.00
VI	17±0.56	12±0.56	27±0.56
VIII	18±0.00	12±0.56	28±0.00
Daktarin® oral gel	20±0.56	0.00	0.00

SEM - Standard error of the mean, Diameter of cork borer = 10 mm

The viscosity of gel I was just about that of the standard marketed oral gel, Daktarin® (Table 7). Therefore, the absence of antimicrobial activity was due to the concentration of extract (8 %w/w) used in the formulation of the product. When the concentration of the extract was increased to 10 %w/w, all the gels produced had antibacterial and antifungal activities except gel III, perhaps due to its high viscosity (80 cp) which may have hindered the release of the active contents.

Comparing the antimicrobial activity of the formulations, only gels VI and VIII (both containing 20 % w/v HPMC as gelling agent) showed activity against all the three organisms susceptible to the extract alone. Formulations II, IV and V showed antimicrobial activity against *Candida albicans* and *Staphylococcus aureus* only. Considering the antifungal property of the preparations, gels V, VI and VIII were comparable to Daktarin® oral gel. Formulations II and IV were excluded for further studies due to their low antifungal activities.

Effect of storage time on viscosities and pH of formulations V, VI and VIII were evaluated for eight weeks as an index of stability of these gels. Stability studies are conducted at all stages of product development cycle with the aim of developing products that are stable, safe and efficacious. Gel V had its viscosity increasing gradually from 70 cp to 75 cp on the 8th week. This viscosity change may affect its antimicrobial potency due to potential delay in the release of its contents. Gel VI had a consistent viscosity of between 66 cp to 67 cp for the 8 week period of study. Similar stability profile was observed with gel VIII which contains white emulsion colour in addition to other ingredients.

A comparative study of pH changes as a function of time revealed only slight fluctuation for all the 3 formulations. There was however a consistent reduction of the pH of gel V up to the 8th week, further reducing its original pH. This raises questions on the suitability of xanthan gum as a gelling agent for this formulation. The pH fluctuations of gels VI and VIII were all within oral cavity optimal pH range of 5.2 – 5.8 and comparable to that of Daktarin® oral gel for the period of study Both herbal extract gels demonstrated potent antimicrobial activity against *Candida albicans* comparable to Daktarin® oral gel when their zones of growth inhibition were compared after the period of study. This showed that using 20 %w/v HPMC as a gelling agent, a product which has similar characteristics as Daktarin® oral gel could be obtained. Addition of white emulsion colour in the formulation of HPMC gel also improved the appearance of the product.

Formulation of pessaries

The displacement values of the ethanolic extract with respect to glycerogelatine and theobroma oil containing 10 %w/w beeswax were found to be 0.51 and 1.25 respectively. The displacement values were used to calculate the amounts of base required for the formulations.

Quality properties of pessaries

The pessaries of both bases were oval in shape, brown in colour (colour of extract), and have smooth surfaces with no air paces when cut open.

Table 9: Quality control parameters of the pessaries (n=3)

Parameter	Glycerogelatine pessaries	Theobroma oil pessaries
Mean weight (g)	1.577	2.075
Maximum %deviation from mean weight	1.88	3.78
Mean disintegration time (min)	19.17±0.76	8.83±0.29
*Mean zone of inhibition (mm)	20.67±0.58	ND

**Candida albicans as test organism; ND = not determined*

Results (Table 9) of the pharmaceutical and microbial evaluations on the pessaries showed maximum percentage deviations of 1.880 % and 3.78 % from the mean weights for glycerogelatine and theobroma oil pessaries respectively though both deviations were acceptable per BP standard, [41] the higher value obtained with the theobroma oil is currently not understood.

Pessaries of both bases passed the disintegration test.[41] (Table 9). Easy disintegration of pessaries could lead to quicker extract release and possible absorption, however, relatively fast disintegration of pessaries can lead to leakage of drug from the vagina leading to reduction of therapeutic effectiveness and patient acceptance and compliance. With relatively high disintegration time the drug has the advantage of staying in the site of action for a long time, prolonging its activity when local action is required. However, such slow disintegration could lead to delay in onset of therapeutic action especially when systemic action is required. Beeswax was added to the theobroma oil base as a hardening agent because using theobroma oil alone the extract produced a pessary with very low melting point and sticky to touch. The 10 % w/w was chosen as the optimum quantity after careful experimentations and analysis.

The antifungal activity of the pessaries with glycerogelatin base against *Candida albicans* showed good antifungal activity as demonstrated by the mean zone of growth inhibition (Table 9). This shows that the pessaries can be used in the treatment of *Candida albicans* infections for its local effect. The extract content per unit dose in all formulations was based on that of G-Rea® herbal mixture which is already on the market.

CONCLUSIONS

In summary, extraction of G-Rea® powder using ethanol gave a higher percentage yield. The extracts contained the same bioactive compounds including tannins, alkaloids, glycosides etc. except flavonoids which was not present in the aqueous extract. Both extracts had antimicrobial activity against *Candida albicans, Staphyloccocus aureus* and *Bacillus subtilis*.

The ethanolic extract was successfully formulated into oral gels and vaginal pessaries. The use of 20 %w/v HPMC as gelling agent and 10 %w/w of the extract and white emulsion colour gave a gel formulation that was stable and showed antimicrobial activity. Pessaries formulated with glycerogelation base were superior in their weight variation profile and disintegration profile compared to those formulated with theobroma oil. Further studies would have to be untaken to isolate the bioactive compounds of the herbal mixture for further investigations.

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