DEVELOPING SUITABLE PHARMACEUTICAL DOSAGE FORMS FOR G-REA HERBAL POWDER

By

YAA GYAMFUAA OSEI

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Pharmaceutics, KNUST. This work has not been submitted for any other degree.

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YAA GYAMFUAA OSEI (20254433) Date

…… ……

MR SAMUEL L. KIPPO (Supervisor) Date

…… ……

PROF K. OFORI - KWAKYE (Head of Department) Date

DEDICATION

This thesis is dedicated to my dear husband Oscar Osei Owusu and my lovely children, Emma, Akua Serwah, Yaa Owusuaa and Elvis, for their sacrifice, love, prayer and support throughout this period.

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ABSTRACT

There is increasing awareness and general acceptability of the use of herbal drugs in today's medical practice. According to the World Health Organisation (WHO), about 80% of the world's population depend on herbal medicines for their primary healthcare needs. However, the use of these medicines is sometimes hampered by lack of definite and complete information about their composition and efficacy. Again patient compliance is affected by the characteristics of the herbal medicine such as the taste, and the dosage form in which it is presented. This study sought to develop suitable pharmaceutical dosage forms for G-Rea herbal powder, a local herbal preparation which is available in the decoction form on the market, used to treat candidiasis. Extraction of powder with 70% ethanol gave a higher yield than extraction with water. Aqueous and ethanolic extracts of the powder showed antimicrobial activity against *B. subtillis, Staph. aureus,* and *Candida albicans*. Phytochemical screening showed the presence of alkaloids, tannins, glycosides, saponins etc. The ethanolic extract had good flow properties compared with the aqueous extract. Oral gels of the ethanolic extract were prepared using various concentrations of different gelling agents, namely 3%w/v xanthan gum, 10%w/v pregelatinised potato starch, 20%w/v hydroxypropyl methylcellulose (HPMC), 2, 4 and 6%w/v carboxymethyl cellulose (CMC). Colour, odour, pH and viscosity and antimicrobial activities of the gels were tested for. It was seen that using 10%w/w of the extract, HPMC 20%w/v as a gelling agent and addition of white colour gave a stable formulation with good antimicrobial activity and good appearance. Pessaries of the extract were prepared using glycerogelatin and theobroma oil + 10%w/w beeswax as pessary bases. Pessaries had good appearance and passed disintegration and uniformity of weight tests. Pessaries with glycerogelatin base had good dissolution profiles. Capsules of the extract were prepared and they also passed disintegration and uniformity of weight tests, they also had good dissolution profile.

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CHARPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

Herbal medicines also called botanical medicines or phytomedicines refer to using plant's seed, berries, roots, leaves, bark or flowers for medicinal purposes (Adeshina *et al*., 2011). Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and defend themselves against predators. Many of these phytochemicals have beneficial effects when consumed by humans and can be used to effectively treat some human diseases.

Herbalism is becoming more main stream as improvements in analysis and quality control alongside advances in clinical research have shown their value in the treatment and prevention of diseases. The use of plants for medicinal purposes dates long before recorded history (Ehrlich, 2011). Early humans recognized their dependence on nature for a healthy life and as such depended on the diversity of plant resources for food shelter and medicines (Kunle *et al*., 2012).

Herbal medicines use is common in many African and Asian countries because of the fact that it is available, relatively affordable and is believed to be safe (Grover *et al*., 2002). W H O estimates that about 80% of the population of some African and Asian countries depend on traditional medicines for some aspect of their primary healthcare. Studies in the USA and Europe have shown that their use is less common in clinical settings but have become more recently, as scientific evidence about their effectiveness have become widely available (Erlich, 2011).

For most herbs, the specific ingredients that cause a therapeutic effect is not known, whole herbs contain many ingredients and they likely work together to produce the desired medicinal effect. These components work together to produce therapeutic effects, lessen the incidence of side effects and enhance effectiveness, synergistic action and reduce toxicity (Chhetri *et al*., 2010).

Most herbal medicines are well tolerated by the patient with fewer unintended consequences than pharmaceutical drugs. Herbs typically have fewer side effects and may be safer to use over a long period of time. Herbal medicines tend to be more effective for long standing health complaints that do not respond well to conventional medicines, they have lower costs and are readily available and accessible compared to conventional medicines.

G -Rea powder is a herbal powder consisting of a mixture of various parts of four different plants. The parts are the roots, stem and the leaves. The plants are *Hoslundia opposita, Alstonia boonei, Combretum smeathmanii* and *Securida longepedunculata.*

These herbs individually are used to treat various ailments traditionally, example, *Hoslundia opposita* which belongs to the family Lamiaceae is used traditionally to treat cystitis, gonorrhea,wounds, etc. *Alstonia boonei* which belongs to the family Apocynaceae is widely used to treat malaria, yaws, sores, ulcers, rheumatic pains and many more. The roots and bark of *Securida longepedunculata* belonging to the family Polygalaceae are taken either as powder or infusion to treat chest complaints, inflammation, veneral diseases, etc. Stem and leaves of *Combretum smeathmanii* of the family Combretaceae are used to treat dysentery (GHP, 1992).

Formulation studies involve developing a preparation of the drug which is both stable and acceptable to the patient. Herbal formulation means a dosage form consisting of one or more

herbs or processed herbs in specified quantities to provide specific nutritional, cosmetic and/or other benefits meant for use to diagnose, treat, mitigate diseases of human beings or animals and/or alter the structure or physiology of human beings or animals (Basavaraj *et al*., 2011).

A dosage form is a mixture of active drug component and non drug component or excipients eg solid, liquid etc. Excipients are substances other than the pharmacologically active drug which are included in the manufacturing process. They may help in the transport of the active ingredients to the site of action of the drug, apart from that they may be important for keeping the drug from being released too early in the assimilation process. Some excipients help the drug to disintegrate into particles small enough to go into solution, in order to be absorbed into the blood stream quickly. Some excipients protect products stability so it will be at maximum effectiveness at the time of use, others are for identification of product while others make the product taste and look better. (Rutesh, 2008). Excipients are thus needed in dosage forms to ensure characteristics physical features of the desired dosage forms are obtained and that the therapeutic performance, safety parameters and stability of the active drug substance are not compromised. Excipients improve patient compliance and are a critical and essential component of a modern drug product (Parker, 2009).

Drugs are formulated into dosage form to ensure large scale manufacture, reproducibility of product, accurate dosage, predictive therapeutic response, convenience of prescribing and administration as well as patient compliance with usage directives (Orafidiya, 2009).

1.2. Justification

Herbal medicines are being used by about 80% of the population in developing countries for primary healthcare. Herbal medicines have stood the test of time for their safety, cultural acceptability and lesser side effects. Improvements in analysis and quality control alongside advances in clinical research have confirmed their value in the treatment and prevention of diseases (Kamboj, 2000).

G-Rea is already on the market in the form of a herbal mixture, developing other dosage forms would increase the scope of use and also improve acceptability and patient compliance.

Formulation of G-Rea herbal to pharmaceutical dosage forms other than the mixture will also ensure reproducibility of product quality, accurate doses of medicines, predictive therapeutic outcomes and easy compliance with usage directives. Developing and formulation of herbal medicines will make it acceptable to all classes of people.

1.3 Aim of study

The aim of this study is to develop suitable pharmaceutical dosage forms of G-Rea herbal powder.

1.3.1 Specific objectives

.To determine a suitable method for the preparation of the G-Rea mixture.

.To extract compounds from powder using ethanol and water, and determine the physical characteristics of the extracts.

.To conduct preliminary phytochemical tests on extract and raw powder and also determine the antimicrobial activity of the crude extract on some selected test organisms.

.To formulate an appropriate oral gel, capsule and vaginal pessaries of the extract.

CHAPTER TWO

LITERATURE REVIEW

2.1. DOSAGE FORM

Dosage form is a mixture of active drug components and non drug components or excipients. There are several forms of dosage forms, which can generally be classified as solid dosage form, liquid dosage form and semisolid dosage form.

Drugs are usually not administered as pure chemical substances alone but are almost always given as formulated preparations. These can vary from relatively simple solutions to complex drug delivery systems through the use of appropriate additives or excipients in the formulation (Aulton, 2007). Excipients provide various pharmaceutical functions, these include solubilisation, suspending, thickening, emulsifying, modifying dissolution, improving the compactibility and flavoring drug substances to form various medicines or dosage forms.

Formulation development is the development of bioactive stable and optimal dosage form for a specific administration route. This involves the use of excipients which ensures that the therapeutic performance, the safety parameters and the stability of the active drug substance is not compromised (Oradifiya, 2009).

2.1.1 HERBAL MEDICINES FORMULATION

A herbal medicine formulation is any medicinal product exclusively containing one or more herbs or processed herb in specified quantities as the active ingredients to provide specific therapeutic, nutritional, cosmetic and other benefits (Basavaraj, 2011). These formulations are obtained by subjecting herbal substances to various treatments such as drying, extraction, distillation, expression, fractionation, purification, concentration, fermentation etc., standardizing and then incorporating the appropriate excipients. Extraction of active

ingredients from plants began in the early 19th century when chemical analysis became available, later chemists began making their own version of plant compounds and over the time, the use of herbal medicines declined in favour of synthetic drugs (Ehrlich, 2011). However, recent interest in herbal medicines due to increased and advanced research , safety, availability and lower cost has led to the need to produce more formulations to meet demand (Vasisht and Kumar, 2002). 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 (Musthaba *et al*., 2010). The most common dosage forms of herbal preparations are liquids derived from macerations, infusions and decoctions, with the associated problems of large dose volumes, difficult packaging and poor stability. Solid preparations such as capsules and tablets on the other hand often have higher stability and are easier to standardize which adds to an increase in their therapeutic acceptance, efficacy and product value (Qusaj *et al*., 2012).

Large scale production of herbal medicines which is as a result of commercialization of herbal medicines requires that scientists and manufacturers maintain the quality and safety of these herbs, as such assurance of quality, safety and efficacy medicinal plants and herbal products have become very important (Rajani and Kanaki, 2008).

2.1.2 Herbal extract

Extracts are defined as a concentrated preparation of a liquid, powder or viscous substance ordinarily prepared from dried plant using an appropriate solvent (HaiQiu, 2006).

These are obtained by removing the active constituents from a part of raw herbs often using suitable solvents such as alcohol and water, evaporating all or nearly all the solvent and adjusting the residual mass to a prescribed standard. They may be obtained in powder form,

aqueous form or the tincture form. Most liquid dosage forms are produced from fluid extracts whiles most solid dosage forms are produced from solid extracts. It is these extracts that usually serve as the active ingredients in the formulation of herbal preparations.

2.1.3. Decoctions

Decoction is a method of extraction by boiling to dissolve chemicals, from herbal or plant material, which may include stems, roots, bark and rhizomes. Decoction involves first mashing, and then boiling in water to extract oils, volatile organic compounds, and other chemical substances. Decoction can be used to make teas, coffees, tinctures and similar solutions. It is used for seeds, roots and bark and other parts of the plant that will not release their medicine at lower heat levels. This method of extraction is used in plants whose medicinal properties are not harmed by the application of heat (Freer, 2011). Decoctions are easy to prepare, however, they are not easy to keep from microbial contamination, long term storage is problematic as active principles may be quite unstable, also traditional measurements and directions are not exact. Generally, decoctions are inconvenient and unpleasant herbal preparations to take.

2.1.4. Capsules

Capsules are solid dosage forms in which the drug is enclosed within either a hard or soft soluble container or shell. The hard-shelled capsules are normally used for dry, powdered ingredients or miniature pellets whiles soft shelled capsules are primarily used for oils and for active ingredients that are dissolved or suspended in oil. Capsule shell is an excellent barrier to air, easy to swallow and tasteless, may allow rapid release and flexibility of formulation (HaiQiu, 2006). Capsule shells are usually formed from gelatin , they may however be formed from starch or other suitable substances (USP, 2007). The hard shell capsule sizes range from No.5 which is the smallest to size 000 which is the largest, except for veterinary sizes.

However, size 00 generally is the largest size acceptable to patients. In herbal capsules formulation, dried and ground herbs are placed into gelatin capsules, which dissolve quickly (USP, 2007). Often capsule of herbs are concentrated for more strength and they are easily portable. Excipients commonly used in capsule formulation are diluents, glidants, disintergrants, lubricants and wetting agents. Hard gelatin capsules usually require between one and four excipients. However there are some drugs in capsule form that contains only the active ingredient, which means there is no excipient. Most of the strongest medicinal herbs are quite bitter to taste, so it is easier to get them down when taking them in capsule form.

A capsule should be able to disintegrate in the stomach followed by the dissolution of the contents in the fluids of the gastrointestinal tract, as such disintegration tests and dissolution tests are conducted on capsules.

2.1.4.1 Tests on capsules

A. Disintegration test: Disintegration test is a standardized test which is primarily used as a quality assurance tool to confirm complete disintegration of solid oral dosage forms when placed in a liquid medium under the experimental conditions described in their respective official monographs. Disintegration test neither implies nor tests for the complete solution of the drug or the dosage form. Hard gelatin capsule are fully disintegrated within about 10 minutes (Gupta *et al*., 2009).

B. **Dissolution test:** the dissolution of a drug substance, under physiological conditions, is essential for the systemic absorption of the drug. For this reason, dissolution testing is typically performed on solid dosage forms to measure the drug release from the drug product as a test for product quality assurance and to determine the compliance with the dissolution requirements when stated in the individual monograph (Gupta *et al*., 2009).

C. Uniformity of weight and content test: These are quality control tests used to determine the uniformity in the amount of the drug substance among dosage units. Each capsule must contain the intended drug quantity with little variation among the capsules in a batch. The drug quantity per capsule of average weight is determined analytically and compared to standards as set in the monographs. Weight of the capsule is the quantity of the powder which contains the labelled amount of the therapeutic agent. The capsule weights must conform to the set standards as in the USP or BP (Bhatt and Agrawal, 2007).

2.1.4.2 Advantages of capsule dosage form

Capsules are easy to use because of the fact that they are smooth, slippery and easy to swallow

It is suitable for substances that have bitter taste and unpleasant odour, it is easy to store and transport.

It is more stable than liquid dosage form and minimum excipients is required in their formulation

Capsules are attractive and available in wide range of colours and they are usually of small particle size so that dissolution and absorption in body fluids is faster (Bhatt and Agrawal, 2007).

2.1.4.3. Disadvantages of capsule dosage form

Not suitable for highly soluble substances and highly effloresce nt or deliquescent materials

Special conditions are required for their storage (Bhatt and Agrawal, 2007).

2.1.5. Tablets

A tablet is a pharmaceutical dosage form which comprises a mixture of active substances and excipients usually in powder form, pressed or compacted into a solid dose. Tablets are the most popular dosage form in use today because they are simple and convenient to use. They are cost effective, convenient to dispense in stores and easy for patient to administer, an accurately measured dosage of the active ingredient in a convenient portable package, and can

be designed to protect unstable medications or disguise unpalatable ingredients. Also release of drug from tablets can be controlled by altering the design and content of the formulation and because it is a dry dosage form, it is stable and has a long shelf life. However, preparation of tablets require the use of excipients such as diluents, binders and lubricants to facilitate the manufacturing process and also ensure that resulting tablets have the desired properties. Tablets should be sufficiently strong to withstand handling during manufacture and usage, they should also disintegrate and release the drug in a predictable and reproducible manner (Mattson, 2000).

2.1.6. Gels

Gels are homogenous semisolid preparations usually consisting of solution or dispersion of one or more active ingredients in suitable hydrophilic or hydrophobic bases. They are prepared with the use of suitable gelling agent and are intended to be applied to the skin or certain mucous membranes for protection and/or therapeutic or prophylactic purposes. Gels may contain suitable auxiliary substances such as antimicrobial preservatives, antioxidants and stabilizers. (BP, 1988). The interactions between the liquid vehicle and the colloidal particles are either physical or covalent, the vehicle is continuous and interacts with the colloidal particles within three dimensional network that is formed between adjacent particles. The vehicle may be aqueous, hydro alcoholic, alcohol based or non aqueous. The colloidal particles may be dispersed solids or dispersed polymers (USP, 2007).

2.1.6.1. Oral gels

Oromucosal gels are semi-solid preparations containing one or more active substances intended for administration to the oral cavity and/or the throat to obtain a local or systemic effect (BP, 2007).

Preparations intended for a local effect may be designed for application to a specific site within the oral cavity such as the gums (gingival preparations) or the throat (oropharyngeal preparations). Preparations intended for a systemic effect are designed to be absorbed primarily at one or more sites on the oral mucosa. Mucoadhesive preparations are intended to be retained in the oral cavity by adhesion to the mucosal epithelium and may modify systemic drug absorption at the site of application. For many oromucosal preparations, it is likely that some proportion of the active substance(s) will be swallowed and may be absorbed via the gastrointestinal tract.

Oromucosal preparations may contain suitable antimicrobial preservatives and other excipients such as dispersing, suspending, thickening, emulsifying, buffering, wetting, solubilising, stabilising, flavouring and sweetening agents (BP, 2007). Oral gels should have acceptable physical and chemical parameters and should also be stable over the period of use. These parameters include the viscosity, pH, spreadability, antimicrobial activity etc, under the suitable storage conditions.

Viscosity: Viscosity of a gel formulation directly affects the release of active ingredients from the formulation. High viscosity of a gel formulation negatively affects the release of active substances from the formulation and as such its penetration through diffusion barriers. Low viscosity positively affects the release of medicaments (Prakash *et al*., 2010).

Spreadability: Spreadability of gel tests the ease of applicability of gels on site of action including ability to spread to orifices (Nilkamal *et al*., 2011).

2.1.7. Ointments

Ointments are greasy, semi-solid preparations, often anhydrous and containing dissolved or dispersed medicaments intended for external application to the skin or mucous membranes. Herbal Ointments are used for topical applications and they are made by mixing powdered

herbs into simple ointments of natural petroleum jelly and lanolin, and can be kept indefinitely in small beauty containers or cream jars with lids. The selection of an ointment base in which drugs are to be incorporated for local application should depend on the condition of the patient's skin, the biological effect desired, and the pharmaceutical compatibility of the ingredients with each other and the base (Lippincott and Wilkins, 2006).

Ointment bases are classifies into four major groups, these are hydrocarbon bases, absorption bases, water removable or emulsion bases and water soluble bases (Lippincott and Wilkins, 2006).

a. The hydrocarbon bases or oleaginous bases are the earliest ointment bases which consist of vegetable or animal fat as well as petroleum hydrocarbons. They are expected to provide a film, which resists soap and water yet readily removable by solutions of surfactants.

b. Absorption bases have hydrophilic or water absorbing properties. These bases are generally anhydrous but capable of absorbing several times their own weight of water ultimately forming water in oil type of emulsions. Absorption bases vary in their composition and are usually mixtures of animal sterols with petrolatum. They were primarily developed so as to have a product to which water or an aqueous solution of medicinal substances could be easily added. These bases are usually highly compatible with the majority of drugs used topically (Marriott *et al*., 2010)

c. Emulsion bases are of two types, the water in oil and oil in water, the water in oil emulsion bases act as emollients while the oil in water are non greasy and non sticky and are often used as cosmetics.

d.Water soluble bases are prepared from mixture of low and high molecular weight polyethylene glycols which range in their consistency from liquids to solids. They are non-

volatile, unctuous, inert and possess the ability to form an emollient surface. They neither hydrolyse and deteriorate nor support mould growth (Marriott *et al*., 2010).

2.1.8. Creams

Creams are semi-solid dosage forms containing one or more drug substances dispersed in a suitable base, that is mixtures of oil and water. They are divided into two types: oil-in-water creams which are composed of small droplets of oil dispersed in a continuous water phase, and water-in-oil creams which are composed of small droplets of water dispersed in a continuous oily phase. A cream is a topical preparation usually for application to the skin and also application to mucous membranes such as those of the rectum or vagina (Langley and Belcher, 2012).

2.1.9. Tinctures

A tincture is typically an alcoholic/water extract of plant or animal material or solution of such or of a low volatility substance such as iodine and mercurochrome. It is used when plants have active chemicals that are not soluble in water and/or when larger quantities is prepared for conveience and wanted for longer term storage (HaiQiu, 2006). To qualify as an alcoholic tincture, the extract should have an ethanol percentage of at least 40–60%. In herbal medicine, alcoholic tinctures are made with various concentrations of ethanol with 25% being the most common. Other concentrations include 45% and 90%. Herbal tinctures are not always made using ethanol as the solvent, though this is most commonly the case. Other solvents include vinegar, glycerol, ether and propylene glycol, not all of which can be used for internal consumption. Ethanol has the advantage of being an excellent solvent for both acidic and basic constituents.

2.1.10. Suppositories

A suppository is a drug delivery system that is inserted into the rectum (rectal suppository), vagina (vaginal suppository or pessary) or urethra (urethral suppository), where it dissolves or melts. They are used to deliver both systemically-acting and locally-acting medications. The principle is that the suppository is inserted as a solid, and will dissolve or melt inside the body to deliver the medicine pseudo received by the many blood vessels that follow the larger intestine. Alternative dosage forms for the rectal and/or vaginal route are tablets, capsules, ointments and enemas (Aulton, 2007). Generally suppositories consist of a vehicle in which the drug is incorporated and in some cases additives are coformulated.

A pharmaceutical pessary is used as a very effective means of delivery of pharmaceutical substances easily absorbed through the skin of the vagina or rectum, or intended to have action in the locality, for example against inflammation or infection, or on the uterus (Woolfson *et al*., 2000).

There are two main classes of vehicles or suppository bases, the fatty base and the water soluble or water miscible bases (Lippincott and Wilkins, 2006).

2.1.10.1 Fatty bases

These are naturally occurring triglycerides, they melt at body temperature. examples are

a. **Theobroma Oil** or **cocoa butter** : it is used as a suppository base because, in large measure, it fulfills the requirements of an ideal base. At ordinary room temperatures of 15° to 25°C, it is a hard, amorphous solid, but at 30° to 35°C, ie at body temperature, it melts to a bland, non irritating oil. Thus in warm climates, theobroma oil suppositories should be refrigerated. Theobroma oil is a polymorphic compound as such should not be heated above 35°C when being used as a base in production because if overheated

it will convert to a metastable structure that melts in the 25° to 30°C range. Thus, the finished suppositories would melt at room temperature and not be usable (Lippincott and Wilkins, 2006).

(b) **Synthetic triglycerides:** Consist of synthetic hydrogenated vegetable oils. Their advantage over cocoa butter is that they do not exhibit polymorphism. They are however more expensive.

2.1.10.2. Water miscible bases

Water soluble/water miscible bases are those containing glycerinated gelatin or the polyethylene glycol polymers. They dissolve or disperse in rectal secretions (Lippincott and Wilkins, 2006).

a. Glycerinated Gelatin

Glycerinated gelatin suppositories are translucent, resilient, gelatinous solids that tend to dissolve or disperse slowly in mucous secretions to provide prolonged release of active ingredients. It is a useful suppository base particularly for vaginal suppositories and also suitable for use in a wide range of medicaments including alkaloids, boric acid and zinc. (Shrewsbury, 2013).

Suppositories made with glycerinated gelatin must be kept in well-closed containers in a cool place since they will absorb and dissolve in atmospheric moisture. In addition, those intended for extended shelf-life should have a preservative added, such as methylparaben or propylparaben, or a suitable combination of the two.

b. Polyethylene Glycol Polymers

They are long chain polymers of ethylene oxide and they occur in solid and liquid form, they are also macrogols. They offer rapid release of active pharmaceutical ingredients with minimal

manufacturing issues (McElroy, 2011). They are chemically stable, nonirritating, miscible with water and mucous secretions, and can be formulated, either by molding or compression, in a wide range of hardness and melting points. Like glycerinated gelatin, they do not melt at body temperature, but dissolve to provide a more prolonged release than theobroma oil.

Certain polyethylene glycol polymers may be used singly as suppository bases but, more commonly, formulas call for compounds of two or more molecular weights mixed in various proportions as needed to yield a finished product of satisfactory hardness and dissolution time.

2.1.11. Properties of an ideal suppository base

Ideal suppository base possess the following properties,

i. Melt at body temperature or dissolve or disintegrate in body fluids.

ii. Be inert, non-irritating and non-sensitizing.

iii. Release the medicament readily.

iv. Be compatible with a broad variety of drugs.

v. Be stable on storage and transportation.

vi. Have wetting and emulsifying properties.

vii. Be able to incorporate a high percentage of water in it i.e., a high water number.

viii. Shrink sufficiently on cooling to release itself from mould and should be mouldable by pouring or by cold compression (Lippincott and Wilkins, 2006).

ix. Fatty bases should have acid value below 0.2, saponification value in between 200 to 245, iodine value less than 7 and a small range between melting and solidification points.

2.1.12. Quality standards of suppositories

Odour: odour should be checked as change in odour could be an indication of degradation of the product

Shape: shape of suppositories should be consistent

Surface condition: the surface of suppositories should be checked for cracks, cavities, dullness, bursts etc

Weight: suppositories should be weighed to determine the uniformity of the weights, this will check whether the mould is being well filled and whether there are air bubbles etc

Melting range (melting point) : The release rate of the suppository is related to its melting point. Suppositories should melt at body temperature and should also be stable on storage and during transportation.

Dissolution testing: One of the most important quality control tools available for in vitro assessment is dissolution testing. Dissolution testing is often required for suppositories to test for hardening and polymorphic transitions of active ingredients and suppository bases. Suppositories should comply with the required standards set in the monographs (USP, 2007).

2.2. PREFORMULATION STUDIES

Pre formulation testing is the first step in the rational development of dosage forms of drug substances. It is defined as an investigation into the physical and chemical properties of a drug substance alone and when combined with excipients. The objective of preformulation tests is to develop information useful to the formulator in developing a stable bio available dosage form (Shivanand *et al*., 2009). Pre formulation involves the characterisation of a drug's physical, chemical and mechanical properties in order to choose what other ingredients should be used in the preparation.

2.2.1. PREFORMULATION TESTS ON HERBAL EXTRACTS

Preformulation steps include selection of the herbal extract, determination of the physicochemical characteristics of the extract, determination of phytoconstituents, antimicrobial activity and consequently minimum inhibitory concentrations. It also involves the determination of other activities of the extract such as antioxidant activity and standardisation etc. Again it includes preformulation parameters such as bulk density, tap density, Carr's index, Hausner's ratio and angle of repose (Nasreen and Narayanan, 2011).

2.2.1.1 Determination of physical characteristics of extracts

Physical property is any property that is measurable and whose value describes a state of a physical system. The changes in the physical properties of a system can be used to describe its transformations or evolutions between its momentary states. Physical properties are often referred to as observables.

Physicochemical characteristics of an extract include colour, odour , clarity, pH, extractive value, moisture content, solubility and ash value.

2.2.1.2. Colour, taste and odour

These are physical characteristics of herbal extracts which are easily identified by simple methods as visualisation, touch, smell or taste. Colour is related to the part of the plant from which the extract originated. Odour comes from the essential oil or other compounds whiles taste vary depending on the components of the material, example, alkaloids taste bitter. All these are used for the preliminary identification of the herbal extract (Prakash *et al*., 2010).

2.2.1.3. pH

The term pH refers to the relative amounts of hydrogen in a given chemical environment. It is a measure of the acidity or alkalinity of a given formulation or product. The pH is important in aqueous drug product formulation, especially since it involves drug solubility, activity,

absorption, stability, sorption and patient comfort (Allen and Edmund, 2011). The pH is also related to certain physical characteristics such as the viscosity of some polymers used as gel-forming agents and in suspensions. The rate of hydrolysis of product may vary depending on the pH of the solution. Determination of the pH will give information as to which excipients could be added to a drug to ensure that the product is stable and can be tolerated physiologically (USP, 2007).

Ash values : They constitute the inorganic residues obtained after complete combustion of a drug, it includes soluble ash, acid insoluble ash, etc. They are a criteria to judge the identity and purity of a crude drug **(**Saraf, 2010).

Moisture content: Checking moisture content helps reduce errors in the estimation of the actual weight of drug material. Low moisture suggests better stability against degradation of product.

Extractive values : These are indicative weights of the extractive chemical constituents of crude drug under different solvent systems.

2.2.1.4. Solubility

The amount of substance that passes into solution in order to establish equilibrium at a constant temperature and so produce a saturated solution is called solubility (Aulton, 2007). Solubility is the property of a solid, liquid or gaseous chemical substance called solute to dissolve in a solid, liquid or gaseous solvent to form homogenous solution of the solute in the solvent. The solubility of a substance fundamentally depends on the solvent used as well as on temperature and pressure. The solvent is generally liquid which may be a pure substance or a mixture of two liquids (Savjani *et al*., 2012).

The extent of solubility ranges widely from highly soluble (fully miscible) such as ethanol in water, to poorly soluble , such as silver chloride in water. The term *insoluble* is often applied

to poorly or very poorly soluble compounds. A drug substance administered by any route must possess some aqueous solubility for systemic absorption and therapeutic response (Pakkir *et al*., 2012).

Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for achieving required pharmacological response. Poorly water soluble drugs often require high doses in order to reach therapeutic plasma concentrations after oral administration.

In the preparation of suppositories and pessaries, the solubility of the drug in the base is of much importance. It determines the type of vehicle to be used, as high solubility of active ingredients in base prevents the release of the drug to the site of action (Aulton, 2012).

Table 2.1 .USP(2007) and BP(2007) solubility criteria

Descriptive term	**Part of solvent required per part of solute**
Very soluble	less than 1
Freely soluble	from1 to 10
Soluble	from 10 to 30
Sparingly soluble	from 30 to 100
Slightly soluble	from 100 to 1000
Very slightly soluble	from 1000 to 10,000
Practically insoluble	from 10,000 and over

2.3. FLOW PROPERTIES OF POWDERS

Flow properties of powders are characteristics of powders that are dependent on particle size distribution, particle shape, chemical composition of the particles, moisture and temperature of storage of the powder (Gabaude *et al*., 2001). Knowledge of flow properties is of first importance to characterise and compare active drug substances and their vehicles. Capsule and tablet production machines require materials with free flowing properties to allow regular dosage of the active ingredients and good production performances. Several methods have been developed to assess the flow properties of powders, these include angle of repose, bulk density, tapped density and Carr's index (Gabaude *et al*., 2001).

2.3.1. Angle of repose

The angle of repose or the critical angle of repose of a powder or a granular substance is the steepest angle of descent or dip of the slope relative to the horizontal plane when material on the slope face is on the verge of sliding. Angle of repose is a characteristic related to interparticulate friction or resistance to movement between particles.

There are many different methods of determining the angle of repose. The different methods may produce different values of angle of repose for the same powder. For this reason angles of repose tend to be variable and are not always representative of flow under specific conditions. As a general guide, powders with angles of repose greater than 50° have satisfactory flow properties whereas angles close to 25° correspond to very good flow properties (Aulton and Taylor, 2013). The different methods for determining angles of repose are:

- Fixed height method
- Fixed base method
- Tilting table method

2.3.2 Bulk and tap density measurements

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density of a powder is dependent on particle packing and changes as the powder consolidates. The bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was done (WHO, 2012). Determinations of Bulk and Tapped Densities are methods to determine the bulk densities of powdered drugs under loose and tapped packing conditions respectively. Loose packing is defined as the state obtained by pouring a powder sample into a vessel without any consolidation, and tapped packing is defined as the state obtained when the vessel containing the powder sample is repeatedly dropped a specified distance at a constant drop rate until the apparent volume of sample in the vessel becomes almost constant. Bulk density can also be used to estimate the mass of powder that will fit in small size containers.

A given mass of powder in a measuring cylinder will have an initial volume, V_O. After tapping for some specific amount of time, it attains a final volume, V_f. The change in volume occurring when void space diminishes is known as 'packing down'. An initial density can be calculated by dividing the mass by the initial volume (Vo).this is known as the initial bulk density or fluff or paired bulk density, D_O. The final density can also be calculated by dividing the mass by the final volume (Vf). This is known as the final bulk density or equilibrium or tapped or consolidated bulk density, D_f.

Hausner found that the ratio D_f/D_0 was related to interparticulate friction and such could be used to predict powder flow properties. Hausner showed that powders with low interparticulate

friction had ratios of approximately 1.2, whereas more cohesive, less free-flowing ones had ratios greater than 1.6 (Aulton, 2001).

2.3.3. CARR'S INDEX

Carr developed another method of measuring powder flow from bulk density measurements called the Carr's index. Carr's index is also known as percentage compressibility and is calculated as $\frac{(D_f - Do)}{Df} \times 100$

2.3.4. Importance of flow properties of powders

Flow properties of powders are very useful in the formulation of solid dosage forms. These properties indicate the ability of the powder to flow and the possibility of direct compression of a powder. The bulk densities of excipient and active pharmaceutical ingredients (API) powders will clearly indicate whether a need exists for the improvent of the density of a powdered sample such as dry granulation before the manufacture of tablets. It also gives an indication of the weight of powder or granules required to fill a particular volume in capsule formulation (Aulton and Taylor, 2013).

2.4 PHYTOCHEMICAL SCREENING

Phytochemical screening is a qualitative chemical evaluation of the crude drug (Kunle *et al.*, 2012).

This is the process of tracing the constituents of a plant sample. Analyzing the phytochemicals in medicinal plants provides scientists with insight into how effective plants are medicinally, and understanding how and why they are effective can lead to the development of new medicines (Doughery, 2012). Phytoconstituents are non nutrient plant chemical compounds and are secondary metabolites of plants which are responsible for the protection of the plant against microbial infections, infestation by pests and general survival

of the plant and its interaction with its environment (Kennedy and Wightman, 2011). These secondary compounds are derived from everyday components, but are not central to metabolism, hence their name. Phytoconstituents of plants include glycosides, tannins, alkaloids, terpenoids, saponins etc.

2.4.1. Tannins

Tannins are naturally produced by plants and are polyphenols with a characteristic of binding and precipitating proteins. Tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, as pesticides, and in plant growth regulations. The antimicrobial activities of tannins are well documented (Chung, 1998). The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins. It has also been found that tannic acid and propyl gallate, are inhibitory to food borne bacteria, aquatic bacteria, and off-flavor-producing microorganisms. Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immune responses (Chung, 1998).

2.4.2 Alkaloids

An alkaloid is a type of plant-derived organic compound, generally composed of oxygen, hydrogen, carbon and nitrogen. Many alkaloids can be used for medical purposes, however their effect vary from medicinal to poisonous. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals. Many alkaloids, though poisonous, have physiological effect that renders them valuable medicine against various diseases including malaria, diabetes, cancer, cardiac dysfunction etc. These are also used in local anesthesia and relief of pain (Aniszewski, 2007). Examples are the local anesthetic and

stimulant cocaine, the stimulant caffeine, nicotine; the analgesic morphine; the antibacterial berberine. Many indole alkaloids are known to have antihypertensive properties while quinine, an alkaloid is known to have antimalarial activity (Wink and Roberts, 1998).

2.4.3.Glycosides

Glycosides are non-reducing substances, which upon hydrolysis yield a carbohydrate molecule called glycone and non carbohydrate moiety called aglycone or genin. They are unique molecules used by plants to safely store potentially dangerous compounds, and by animals to eliminate toxins (Braun and Cohen, 2010). A number of plants stock up compounds in the form of inactive glycosides. These chemicals can be activated by enzyme hydrolysis, which results in the detachment of the sugar portion. This makes the sugar portion available for use, many such plant glycosides are used as medications. The molecules have other uses such as use as natural sweeteners, used in as cleaning agents etc (Kren and Martínková, 2001).

2.4.3.1 Flavonoid glycosides

The aglycone in this instance is a flavonoid. In fact, this one is a vast cluster of flavonoid glycosides and some of the examples of flavonoid glycosides include naringin, hesperidin, quercitrin and rutin. Flavonoid glycosides possess antioxidant properties and this is very beneficial for the overall wellbeing of humans. The antioxidant effect of flavonoid glycosides are said to lessen capillary weakness. Among the many benefits attributed to flavonoids are reduced risk of cancer, heart disease, asthma, and stroke (Erik, 2003).

2.4.3.2 Saponins

Glycosides with foaming features are known as saponins. Saponins comprise polycyclic aglycones bound to one or many sugar side chains and the aglycone segment of the glycoside, also known as sapogenin, is a steroid or a triterpene. Saponins possess foaming aptitude and

this is the outcome of the amalgamation of a hydrophilic sugar segment of the glycoside and a hydrophobic sapogenin. Generally, saponins taste bitter and some of them are also poisonous. When wobbled with water, these compounds create permanent foam. The compounds also result in the haemolysis of the red blood cells. Saponin glycosides have a prominent therapeutic benefit as they possess expectorant properties. Saponins bind with bile salt and cholesterol in the intestinal tract. Bile salts form small micelles with cholesterol facilitating its absorption. Saponins cause a reduction of blood cholesterol by preventing its re-absorption (Cheeke, 1998).

2.4.3.3 Anthraquinone glycosides

These glycosides enclose an aglycone group that is derived from anthraquinone. These glycosides are found in aloes, rhubarb and senna. They possess a laxative or purgative property (Bolen, 2012).

2.4.3.4 Steroidal glycosides or cardiac glycosides

In this case, the aglycone segment is a steroidal nucleus. Generally, steroidal glycosides or cardiac glycosides are present in the plant genera digitalis, strophanthus and scilla. As the name of this glycoside suggests, they are used to treat heart conditions such as congestive heart failure and arrhythmia. However, presently medical practitioners prefer other agents than these glycosides to treat conditions like congestive heart failure (Prassas and Diamandis, 2008).

2.4.4 Terpenoids

Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Terpenoids display a wide range of biological activities against cancer, malaria and a variety of infectious diseases. Examples of terpene based

pharmaceuticals are artemisinin, an antimalarial and taxol an anticancer drug (Wang *et al*., 2005).

2.5. Determination of antimicrobial activity and minimum inhibitory concentration (MIC)

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms that they primarily act against. For example, antibacterials or antibiotics act against bacteria whiles antifungals act against fungi. They can also be classed according to their function. Antimicrobials that kill microbes are called microbicidal whiles those that inhibit their growth are called microbiostatic. Disc diffusion or agar diffusion and broth dilution methods commonly used in clinical laboratories to assess microbial susceptibility/resistance to antimicrobials (Jiang *et al*., 2013). The current available screening methods for the detection of antimicrobial activity of natural products fall into three groups, including bioautographic, diffusion, and dilution methods. The bioautographic and diffusion methods are known as qualitative techniques since these methods will only give an idea of the presence or absence of substances with antimicrobial activity. On the other hand, dilution methods are considered quantitative assays once they determine the minimum inhibitory concentrations (Valgas *et al*., 2007).

2.5.1. Minimum inhibitory concentration (MIC))

Minimum inhibitory concentration is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation, or the standard for determining the susceptibility of antimicrobials. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better antimicrobial agent. MIC is generally regarded as the most basic

laboratory measurement of the activity of an antimicrobial agent against an organism. MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the in vitro activity of new antimicrobials (Andrew, 2001).

2.5.2 Agar diffusion method

It is a means of measuring the effect of an antimicrobial agent against microorganisms grown in culture. It involves the movement of molecules from the antimicrobial agent to the microorganisms grown on culture through diffusion. This tests for the effectiveness of the agent against the microorganism. If the compound is effective against the microorganism at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the filter disk, the more effective the compound (Bonev *et al*., 2008).

2.5.3 Broth dilution method

This is a technique in which containers holding identical volumes of broth with antimicrobial solution in incremental concentrations usually geometrically are inoculated with a known volume of microorganisms. This method is also used to determine the minimum inhibitory concentration. In this method, the ability of microorganisms to produce visible growth in a series of broth containing dilutions of the antimicrobial agent is tested. The lowest concentration of the antimicrobial agent that is able to prevent the appearance of viable growth of microorganisms under defined conditions and within a defined period of time is the minimum inhibitory concentration (EUCAST, 2003).

2.6. G-REA HERBAL POWDER

G-Rea Herbal powder is a polyherbal powder made of a mixture of various parts of different plants in equal quantities dried and milled together. Already available in the decoction form on the market, it is being used in the treatment of candidiasis. The components of this powder are *Hoslundia opposita, Alstonia boonei, Combretum smeathmanii,* and *Securida lonepedunculata.*

2.6.1 *Hoslundia opposita* vahl

Figure 2.1. Picture of *Hosludia opposita*. Source: www.plantzafrica.com

Description : Also called orange bird berry plants, and '*Aberewa ninsu'* in Twi, they are herbaceous perennials and sometimes soft shrubs, growing up to 1.2 m high. It belongs to the family Lamiaceae. It has branches and leaves which are opposite or sometimes arranged in threes.. Plants possess minute, white or creamy green-coloured flowers, starting from October to February. Fruits are fleshy, berry-like in shape and attractively orange-red in colour. These plants have a widespread natural distribution and occur in both tropical and subtropical open woodland. These plants are very common throughout tropical Africa in countries such as Senegal, Nigeria, Ghana etc, it is also found in some parts of Southern Africa.

Uses

- Infusions of its leaf are widely used in African traditional medicine for treating various ailments including diabetes (Abbiw *et al*., 2002). Leaves are reported to have a strong unpleasant scent, which is alleged to repel bees and is thus utilized in the collection of honey. Research into the essential oils of the plant showed that it has significant activity against *Aspergillus niger, Acinetobacter calcoacetica, Brochothrix thermosphacta* and *Flavobacterium suaveolens* (Gundidza *et al*., 1992). The root bark of *H. opposita* is used to

treat malaria as it was found to contain compounds that inhibit the growth of multidrug resistant strain of *Plasmodium falciparum* (Achenbach *et al*., 1992).

Traditionally, it is used as a purgative, diuretic, antibiotic and antiseptic for the treatment of gonorrhoea, cystitis, coughs, fever, wounds, convulsions, sores, mental disturbances, abdominal pains, malaria; snake bites and for the relief of swellings, herpes, conjuctivitis, etc (Achenbach *et al*., 1992).

The Ghana Herbal Pharmacopoeia also states that it is used in cases of Colds, cough, convulsion, diabetes mellitus, fever, jaundice, *Herpes zoster*, purgative; sore throat; wounds (GHP, 1982).

2.6.2 *Alstonia boonei de wild*

Figure 2.2. Picture of *Alstonia boonei de wild.* Source: www.hindawi.com

Alstonia boonei is a herbal medicinal plant popularly known as "Onyame dua" meaning God's tree in the Ghanaian language Twi. It is a giant tree in most of the evergreen rain forests of tropical West Africa. The plant thrives very well in damp riverbanks (Adotey *et al*., 2012). It belongs to the family apocynaceae. It is a decidous tree of up to 45m high and about 1.2m in diameter. Its creamy white wood is utilized for light constructions in interior joineries, furniture, household equipments, sculptures, boats, boxes, matches, pencils, moldings and plywood. It serves as shade tree for people and also coffee, tea and banana plantations (Moronkola and Kunle, 2012), (Palla, 2005). Within West Africa, it is considered as sacred in some forest communities, consequently the plant parts are not eaten (Adotey *et al*., 2012).

The plant parts have been traditionally used for its antimalarial, aphrodisiac, antidiabetic, antimicrobial, and antipyretic activities, which have also been proved scientifically. *Alstonia boonei* is for the treatment of malaria, fever, intestinal helminthes, rheumatism and hypertension.

The major phytochemicals in the stem bark are saponins, alkaloids, tannins and cardiac glycosides (Fasola and Egunyomi, 2005).

In local markets in West and Central Africa it is often amongst the most common plant materials sold as crude drugs. A bark decoction is widely used to treat malaria, typhoid fever, gonorrhoea, yaws, asthma and dysentery, and is also applied to sores, ulcers, snakebites, rheumatic pain and toothache. A maceration of the bark is taken to treat jaundice, cough and sore throat, and is applied externally to treat various skin complaints. The bark is also used as an anthelmintic. The latex is applied to snakebites, skin complaints and swellings caused by filaria infections, and in concoction to treat fever. The leaves are applied topically to reduce oedemas and to treat sores.

2.6.3 *Combretum smeathmanii G Don*

Figure 2.3. picture of *Combretum smeathmanii* G.Don. Source: *www.trichilia.n*

Combretum smeathmanii G.Don (Combretaceae) is a scandent shrub widely used by traditional healers in Ghana and is indicated for the treatment of wounds, boils among other conditions (Kisseih *et al*., 2013).

2.6.4 *Securida longepedunculata*.fresen

Figure 2.4. Picture of *Securida longepedunculata*.fresen. Source: *www.plantzafrica.com*

Description

Securidaca longepeduculata is a violet tree which is small to medium-size, it grows up to 6 m high, with characteristic pale grey, smooth bark. Leaves are variable in size and shape, alternate, often in clusters or crowded on dwarf spur branchlets which are sometimes spine-tipped. They have very fine hairs when young but they lose them as they mature. Flowers are sweetly scented, in short bunches, pink to purple and are produced in early summer. They are about 10 mm long and are each borne on a long, slender stalk (peduncle). Terminal and axillary sprays are about 30-50 mm long, appearing with the very young leaves. The fruit is round, with a distinctive membranous wing up to 40 mm long, purplish green when still young, becoming pale straw-coloured, and can be seen between April and August, it belongs to the family polygaceae.

Uses

The violet tree is the most popular of all the traditional medicinal plants in South Africa and is used for almost every conceivable ailment. The roots are extremely poisonous, smell like wintergreen oil and contain methyl salicylate which may partly indicate why they have a wide diversity of uses, such as arrow poison in some parts of Africa including West Africa. The roots and bark are taken orally either powdered or as infusions for treating chest complaints, headache, inflammation, abortion, tuberculosis, infertility problems, venereal diseases and for constipation. Toothache can also be relieved by chewing the roots. Mixed roots of the violet tree and dwarf custard apple are used to treat gonorrhea. Powdered roots or wood scrapings are used to treat headache by rubbing them on the forehead, while infusions from the roots are used to wash tropical ulcers. It is also believed that many African people use the powdered violet tree roots as a sexual boost for men. The bark is used to make soap, fibre for fishing nets, baskets and strong threads that are used to sew cloth. In Zimbabwe, the roots are used to treat people who are believed to be possessed by evil spirits, for snakebite as well as for coughs when pounded with water and salt *(*Avhurengwi, 2006).

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Herbal materials and excipients

G-Rea herbal powder and G-Rea herbal mixture were obtained from Osei Herbal clinic, Kumasi. Carboxymethylcellulose, Hydroxypropylmethylcellulose (HPMC), aspartame were obtained from UK Chemicals, Kumasi. Xanthan gum, potato starch, arachis oil, theobroma oil base, lactose, beeswax and gelatin powder were obtained from the Chemical Store of the Department of Pharmaceutics, KNUST, Kumasi.

3.1.2. CHEMICALS AND REAGENTS

Ethanol 96%, benzoic acid, concentrated hydrochloric acid (conc HCl), MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, were obtained from the Chemical Stores of the Department of Pharmaceutics and the Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST

3.1.3. EQUIPMENT AND APPARATUS

Eutech pH meter (pH 510, pH/mV/^{0}C meter), porcelain mortar and pestle, Analytical balance (Adam Equipment), Erweka Dissolution Apparatus, (Type DT 6, GmbH Heusenstamm), Erweka disintegration apparatus (Erweka ZT 120), Bunsen burner, water bath, hot air oven (Sany, OMT Oven, Gallenkamp,UK), laminar air flow cabinet (Ohaus Corporation, Pine brook NJ), Gallenkamp Plus II cooled incubator , No 5 cork borer, spatula , Reprospher HPLC apparatus ($C_{18,}$ 5µm) etc.

Brookfield Viscometer (Brookfield Engineering Lab Inc., Middleboro, MA, USA), Sartorius Electrical Balance and general glassware.

3.1.4. Test organisms

E.coli ATCC25922, *Pseudomonas aeruginosa* ATCC 4853, *Staphylococcus aureus* ATCC25923, *Bacillus subtillis* NTCC10073 and *Candida albicans* (clinical strain).

3.2. METHODS

3.2.1. PREPARATION OF EXTRACTS

3.2.1.1. Preparation decoctions (herbal mixtures)

Formula for the preparation of the decoctions was based on formula given by the producers of G-Rea herbal mixture on the market. In this, 50g of powder was weighed into a one litre container and 660ml of water was added, it was slowly boiled for one hour and allowed to cool to room temperature. It was filtered and benzoic acid and aspartame were added to the filterate as preservative and sweetener respectively, enough water was added up to 660ml. The resultant decoction was bottled, well covered, labeled as product 'A' and stored at room temperature for further use. Another decoction was prepared using the above method but this time without benzoic acid the preservative and labeled product 'B'. In a third preparation, the decoction was prepared but a sweetener was not added it was labeled product 'C'. The taste, colour, odour, clarity and pH of the products were recorded. The antimicrobial activities of the products were also tested and compared to the activity of G-Rea herbal mixture sold on the market.

Table 3.1. Formula for product A.

Ingredients	Quantities
G –Rea powder	50g
Aspartame	0.1g
Benzoic acid	0.01g
Water to	660ml

Table 3.2. Formula for product B

Ingredients	Quantities
G-Rea powder	50g
Aspartame	0.1g
Water to	660ml

Table 3.3. Formula for product C

Ingredients	Quantities
G-Rea powder	50g
Benzoic acid	0.01g
Water to	660ml

3.2.1.2. Preparation of aqueous extracts (dried extract)

Fifty grams of the powder was boiled in one litre of water for one hour and allowed to cool to room temperature, it was filtered and the filtrate dried in hot air oven at 45℃ for four days, the yield of the extract was recorded.

G-Rea herbal mixture sold on the market was obtained and dried slowly in a hot air oven at 45℃ for four days, the powdered sample obtained was weighed and phytochemical tests were conducted on it.

3.2.1.3. Extraction by cold maceration

A weight of 100g of G-Rea powder was weighed into a clean two litre container and one litre of 70% ethanol was added, it was stirred intermittently for 72hours and filtered. The residue was further extracted with one litre of 70% ethanol for another 48 hours to ensure exhaustive extraction of the powder. The filtrate was slowly dried in an oven at 45℃ for 48 hours. The yield of the extract was weighed with an electronic balance and then recorded. The extract was ground with a mortar and pestle to obtain a smooth powder.

3.2.2. Determination of physicochemical characteristics of extracts.

3.2.2.1. Physical characteristics of extracts

The colour of the powdered extracts and decoctions were observed in a well illuminated room and recorded. The odour, taste, pH, clarity and texture of the various extracts were also recorded.

3.2.2.2. pH

One gram of each of the dried extracts was weighed and 5 ml of water was added and left overnight, it was filtered and the pH of the filtrate was determined using the Eutech pH meter which had previously been calibrated. The pH of the various decoctions prepared were also

determined, in this about 5 ml of each sample was taken into a 10 ml beaker and the pH taken using the Eutech pH meter. All test were carried out at room temperature.

3.2.2.3. Solubility testing

The solubility of the dried extracts in water and ethanol was tested.

In the water solubility testing, 5g of each of the extracts was weighed into 100ml of water and well shaken occasionally for the first 6 hours, they were then kept undisturbed for 18 hours at room temperature, each was filtered and 25ml of the filtrate was taken and put in pre-weighed crucible. It was dried in an oven at 45℃ for about 3 hours and weighed. The above procedure was used to determine the solubility of the extracts in ethanol. Extract in ethanol was dried for one hour.

3.2.2.4. Moisture content determination

Two grams of each of the extracts was placed into a porcelain crucible which had previously been dried to a constant weight and weighed. It was 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 interval until a constant weight was reached after four consecutive readings. The weight of the crucible and extract was recorded, the determination was done in triplicate. The moisture content or loss on drying was expressed as a percentage of the extract. (Archana *et al*., 2010).

3.2.2.5. Bulk and tapped density determination

Ten grams of the powdered ethanol extract was weighed and poured through a funnel into a 100 ml measuring cylinder. The cylinder was then lightly tapped twice to collect all the powder sticking on the wall of the cylinder. The initial volume, V_o was recorded. The cylinder was tapped from a height of 2.0 cm on a wooden bench top 100 times to attain a constant volume reading from the cylinder, V_f. The initial density was calculated as the initial bulk density or paired bulk density, i.e. D_o = mass/V_o. The final density was also calculated as the

final bulk density or tapped or consolidated bulk density, ie D_f = mass/ V_f (Trivedi *et al*., 2008).

Hausner's ratio: The ratio $\frac{D\,f}{Do}$ calculated as the Hausner's ratio.

Carr's index: Carr's index also known as percentage compressibility was then calculated as

Carr's index = [$\frac{\text{Tapped density } (D_f) - \text{bulk density } (D_o)}{\text{Tapped density } (D_f)}$] x 100 (Trivedi *et al*., 2008).

3.2.2.6. Angle of repose

The angle of repose was also performed using the fixed height method. A funnel was clamped with its tip 10cm above a paper placed on a flat horizontal surface. The powdered extract was carefully poured through the funnel to form a cone and carefully continued until the apex of the cone was just about to slide. The base of the cone was marked and the height of the cone was also measured. The angle of repose was calculated from the height of the cone (h) and the radius of its base (r) using the relation, $\tan \theta = h/r$ (Ejikeme, 2008).

3.2.3. Phytochemical screening

3.2.3.1. Test for alkaloids

Each of the samples was extracted with 5 ml of ammoniacal alcohol, it was filtered and the filterate evaporated to dryness. The residue was extracted with 1% H_2SO_4. It was filtered, and the filterate rendered distinctly alkaline with dilute ammonia (NH_3) solution, it was shaken with 10 ml of chloroform ($CHCl_3$) in a separating funnel and the chloroformic extract separated. The chloroform was evaporated off and the residue dissolved in 2 ml 1% H_2SO_4 . One drop of Mayer's reagent was added to the extract and the results were observed. Again, one drop of Dragendoff's reagent was added to the extract and the results observed (Devmurari, 2010).

3.2.3.2. Test for tannins

A weight of 0.5g of each of the extracts was boiled with 25ml of water for 5 minutes in a 50 ml beaker, it was cooled, filtered and the volume adjusted to 25ml. To 1ml of the extract, 10 drops of 1% Lead acetate solution was added, the colour of the precipitate formed was recorded.

3.2.3.3. General test for glycosides

A weight of 200mg of each of the extracts was put in 20ml test tube, extracted with 5ml of dilute HCl on a water bath for 2 minutes, it was filtered and the filterate rendered distinctly alkaline by adding 5 drops of 20% NaOH and tested with a pH paper. To the filterate, 1ml of Fehlings solutions A and B were added and heated on a water bath for 2 minutes. The colour of the precipitate produced was noted (Egwaikhide and Gimba, 2007).

3.2.3.4. Test for saponins

A quantity of 200mg of the extracts was shaken vigorously with 10ml of distilled water and allowed to stand for about 2 minutes. The extract was then filtered and the filtrate shaken

vigorously. The appearance of froth on the surface which does not break readily upon standing indicates the presence of saponin glycosides (Devmurari, 2010).

3.2.3.5. Test for anthraquinones (Borntrager's Test)

A quantity of 200mg of each of the extracts was boiled with 10 ml of dilute H_2SO_4 for five minutes, it was filtered whilst still hot, it was cooled and the filterate was shaken with 5 ml of chloroform in a separation funnel and the chloroform layer was separated. The chloroformic layer was shaken with 2.5 ml of dilute NH_3. The colour of the solution was observed (Ayoola *et al*., 2008).

3.2.3.6. Test for flavonoids

An aqueous extract of each of the samples were prepared by maceration and filtered. A strip of filter paper was dipped in the liquid extract and dried. The paper was exposed to ammonia solution and the observation recorded (Mamta and Jyoti., 2012) .

3.2.3.7. Test for steroids

A chloroformic extracts of the powders were prepared, to 0.5ml of the extract, about 2 ml of concentrated H_2SO_4 was added carefully down the side of the tube to form a lower layer. The colour at the interface was observed and recorded (Devmurari, 2010).

3.2.3.8. Test for terpenoids

A chloroformic extract of each of the extracts was prepared, and to 5ml of each extract, acetic anhydride was added, then 5 ml of concentrated H_2SO_4 was carefully added down the side of the tube to form a lower layer. Colour at the interface was observed and recorded (Edeoga *et al*., 2005)

3.2.4. HPLC Analysis of ethanolic extract

3.2.4.1. Conditions :

Brand of column : Reprospher

Stationary phase: C18

Mobile phase : methanol: water (20:80)

Flow rate: 1ml/min

Injection volume : 20 microlitres

Detection wavelength : 283nm

Temperature : ambient

3.2.4.2. Method :

A solution of the powdered sample was prepared by dissolving 0.1g of the powder in 100ml of water to obtain a concentration of 0.1%w/v. The mobile phase was prepared by shaking thoroughly a mixture of 100ml methanol and water (in the ratio of 20:80 respectively) to a homogenous mixture. The stationary phase was conditioned until equilibration was reached. 0.1%w/v of the sample was then injected to give an appropriate chromatogram.

Method development started with 50:50 methanol : water as mobile phase and continuously varied until the above composition as it gave a better chromatogram.

3.2.5. TESTS FOR ANTIMICROBIAL ACTIVITY OF EXTRACTS

3.2.5.1. Agar Diffusion method

In-vitro antimicrobial activity of the extracts was evaluated using the agar well diffusion technique. Nutrient agar was used as the medium. The test bacteria used are *Pseudomonas aeruginosa, Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtillis* and the fungi used was *Candida albicans.* 20 tubes of 20ml nutrient agar were each melted and stabilized at 45℃ for 15 minutes in a reciprocal water bath shaker. The stabilized agar were seeded with 0.1ml of a 24hour broth culture of the test organisms. The seeded agar were rolled in the palms to allow for thorough mixing of the organisms and then poured aseptically into sterilized petri dishes and allowed to set at room temperature. Wells were bored using a sterile borer size 5 with internal diameter of 10mm and labeled appropriately. Each well was filled with the different concentrations of the ethanolic and aqueous extracts of the powder, the concentrations prepared were 10%w/v, 5%w/v, 2.5%w/v and 1.25%w/v. 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.

In the antifungal activity determination using *Candida albicans*, the above procedure was used but the culture media used was Sabouraud agar. All tests were done in triplicates.

3.2.5.2. Determination of antimicrobial activity of decoctions

In this determination, the method used was similar to that of the ethanol extracts, the wells created in the agar were filled with the various decoctions and the activities of the products were compared with that of the G-Rea herbal mixture on the market.

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

Calculated volumes of the extracts in test tubes were serially diluted with double strength nutrient broth and 0.1ml of 24 hour broth culture of the test organisms were added to each tube. Required volume of sterile water was then added to make it up to the required volume of 2ml, 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℃ for 24 hours. To determine bacterial growth, 1ml of 125mg/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 whiles 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.

3.2.6. PREPARATION OF ORAL GELS

Table 3.4. Formulation of oral gels

	Formulations							
Ingredients	I	II	III	IV	V	VI	VII	VIII
G-REA extract (g)	4	5	5	5	5	5	5	5
Glycerol (g)	2	2	2	2	2	2	2	2
Aspartame (g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cocoa flavour (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Orange flavour (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
White emulsion colour (g)								0.2
Orange colour (g)							0.2	
2%w/w CMC (qs)	50							
4%w/w CMC (qs)		50						
6%w/w CMC (qs)			50					
10%w/w PPS (qs)				50				
3% w/w XG (qs)					50			
20%w/w HPMC (qs)						50	50	50

CMC: carboxy methylcellulose, XG: Xanthan gum, PPS: Pre gelatinised potato starch,

HPMC: Hydroxypropyl methylcellulose.

3.2.6.1. METHOD

Powdered ethanol extract was used in the preparation. In the preparation of oral gels, concentrations of the herbal extract were selected based on the minimum inhibitory concentration of the extract in order to obtain a product that possess antimicrobial activity. Different polymers were tested as gelling agents in order to obtain a product which the active ingredient and the gelling agent were compatible and stable. The concentrations of extract used were 8% and 10% . The gelling agents used were 3% xanthan gum (XG), 20% w/w hydroxypropylmethylcellulose (HPMC), carboxymethylcellulose (CMC) of concentrations 2%, 4% and 6%w/w, and 10%w/w pregelatinised potato starch (PPS). These concentrations of the gelling agents were selected in order to get a product whose viscosity is close to that of Daktarin oral gel, the reference product. Other ingredients used were glycerol as a preservative, aspartame as a 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 indicated in table 3.4. The calculated quantity of the powdered extract was accurately weighed, dissolved in ethanol and mixed with aspartame and glycerol in a pre weighed mortar, it was well titurated to form a smooth paste. Enough of the prepared gelling agents were added and well mixed to obtain a smooth gel and to the required weight of 50 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 ℃) . The physical characteristics of the gels were observed. Viscosity and pH of formulations were tested weekly

for 8 weeks, and antimicrobial activities were also tested, results were compared with that of Daktarin oral gel which is an oral gel on the market.

3.2.6.2. Determination of antimicrobial activity of the gels

The antimicrobial activity of the various gels prepared was determined using the agar diffusion method. In this, the various gels were introduced into wells created in nutrient agar which had previously been inoculated with 24 hour broth culture of the various susceptible test organisms. The antimicrobial activity of the products were recorded. The activity of the products against *Candida albicans* were compared to activity of Daktarin oral gel. The antifungal efficacy study against *Candida albicans* was determined by agar diffusion method employing cup plate technique (Harish *et al*., 2009).

3.2.6.3. pH determination of gels

The pH of various gel formulations were determined by using a pH meter which was calibrated before each use with standard buffer solutions at pH 4 and 7. One gram of each gel was dissolved in 100 ml distilled water and stored at room temperature for two hours and the pH of the resultant solutions were determined. The measurement of pH of each formulation was done in triplicate and average values were calculated.

3.2.6.4. Viscosity determination of gels

The determination of viscosities of the prepared 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℃). Each gel formulation was in turn placed in the sample cup and allowed to stand until it reached room temperature (30℃) before the viscosity was determined. Evaluations were done in triplicates and average viscosities were calculated (Harish *et al*., 2009).

3.2.7. VAGINAL PESSARIES PREPARATION

3.2.7.1. Determination of the displacement values of extract in theobroma oil + 10%w/w beeswax base and glycerogelatin base

Suppository moulds used in preparation of the pessaries were the 2 g suppository mould. The moulds were cleaned and lubricated. The lubricants used were liquid paraffin for glycerogelatin base and soap spirit for theobroma oil base. 20 g of the base was weighed, melted and poured into the mould. Five of the resulting pessaries were weighed and recorded as weight of blank suppository. In another formulation, 10%w/w of extract was incorporated into the base and five of the resultant suppositories also weighed and recorded as weight of medicated suppository. Displacement value was calculated by this equation

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

3.2.7.2. Preparation of vaginal pessaries

Table 3.5. Formula for the preparation of 10%w/w G-Rea pessaries with theobroma oil +10%w/w beeswax base

Ingredient	Quantities
G-Rea powder	6g
Theobroma oil	54g

Table 3.6. Formula for the preparation of 10%w/w G-Rea pessaries with glycerogelatin base

Ingredient	Quantities
G-Rea extract	7.2g
Glycerogelatin base	64.8g

3.2.7.2.1. Method

Thirty suppositories of each of the two bases were prepared. A clean dry mould was obtained and lubricated with the aid of a cotton wool. With the aid of an electronic balance, the appropriate weights of the ethanolic extract of the powder were weighed. The base was heated in a spouted pan over a water-bath until just melted. The medicament was levigated into a little of the base on a warm tile and then stirred into the rest of the base until a homogenous mixture was obtained. The melted mass was then poured into the mould until it over flowed, the mould was then refrigerated for about 10 minutes. When cooled, any excess base was scraped from the top of the mould with a warm spatula, the mould was opened and the preparations removed and packed. The bases used were glycerogelatin base and theobroma oil base.

3.2.7.3. EVALUATION OF PESSARIES

3.2.7.3.1. Physical characteristics of pessaries

Pessaries were physically observed for shape, colour and texture. They were cut open to observe the presence of bubbles or otherwise.

3.2.7.3.2. Disintegration tests on pessaries

The apparatus used in this test was the BP disintegration apparatus for tablets and capsules. A pessary was placed in each of the six tubes on the disintegration apparatus and the time taken for melting or disintegration of all six pessaries maintained at 37℃ in 1000ml distilled water was recorded.

3.2.7.3.3. Uniformity of weight tests on pessaries

Twenty (20) pessaries from each batch of pessaries were randomly selected and weighed together; the mean weight of the pessaries was calculated as A. The pessaries were then weighed individually and recorded as weight B. The weight of each pessary was subtracted

from the mean pessary weight (of twenty pessaries) (A-B) and the percentage deviation of each pessary from the mean was also calculated using the formula $\frac{A-B}{A} \times 100$.

3.2.7.3.4. Determination of the antimicrobial activity of pessaries of glycerogelatin base

The agar diffusion method was used in this test. In this one of the pessaries was slowly dissolved in about 1ml warm water. Sabourauds agar was melted and stabilised at 45℃ for 15 munites. It was seeded with 24 hour broth culture of *Candida albicans* and allowed to set. Wells were created in the agar with number 5 cork borer and the wells were filled with the dissolved pessaries. The agar was incubated at 37℃ for 24 hours and the zones of inhibition were recorded

3.2.7.4. DISSOLUTION TESTS ON PESSARIES

3.2.7.4.1. Determination of maximum wavelength of absorption of extract.

One gram of the crude dried ethanol extract of G-Rea was weighed and dissolved in a quantity of 0.1M HCl in a 100ml volumetric flask. More of 0.1M HCl was added to the 100ml mark and the solution shaken. Serial dilutions of the solution were then made to obtain several concentrations (0.0001, 0.001, 0.010, 0.1 %w/v). The solutions were scanned to obtain the maximum wavelength of absorption using a UV- Vis Spectrophotometer.

3.2.7.4.2. Calibration curve for G-Rea extract.

Solutions of concentrations (0.025, 0.015, 0.0125, 0.01, 0.0075, 0.0050, 0.0025%w/v) were prepared from the crude dried G-Rea extract and their corresponding absorbance were recorded at a wavelength of 283nm using UV- Vis Spectrophotometer. A calibration curve showing the relationship between concentration and absorbance was plotted and the equation and correlation values of the curve generated from the scatter plot.

3.2.7.4.3. Dissolution testing

The procedure described by the BP 2007 was used.

The experimental conditions adopted for the dissolution of the pessaries were as follows

Medium	900ml 0.1M HCl
Basket speed	50rpm
Sampling times	5, 15, 30, 45 and 60 minutes
Temperature	37℃ ± 0.5℃

The six vessel dissolution apparatus was used. The water bath was filled to the maximum mark and the compartments labelled A, B, C, D, E and F. The round bottom beakers were each filled with 900ml of 0.1M HCl, placed in their respective compartments and held firmly in the bath. The seventh beaker was also filled with the dissolution medium and put in its position. The thermostat was set at 37℃. The height of the basket was set at about 2cm above the bottom of the beakers and revolutions set at 50rpm. The thermostat was switched on and the water bath and its contents were allowed to reach temperature equilibrium of 37℃ ± 0.5℃. The time was set at 0.00 and samples introduced into the 6 consecutive round bottom beakers at 4 minute interval. At 5 minutes, 10ml of the dissolution medium was withdrawn from vessel A, and discarding the first few millilitres, it was filtered immediately into a test tube. 10ml of fresh dissolution medium was withdrawn from the seventh beaker to replace the 10ml withdrawn from the beaker. Filtrate was then diluted five times with 0.1M HCl. The procedure was repeated at times 15, 30, 45 and 60 minutes for Vessel A. Vessels B, C, D E and F were also taken through the same process.

The diluted filtrate were analysed by UV Vis spectrophotometer at a wavelength of 283nm using a 1cm cell and 0.1M HCl as the reference sample. Using the equation obtained from the calibration curve, the concentrations of extract in samples taken at dissolution times were calculated and the percentage release values were then calculated. A plot of cumulative percentage drug released against time was established.

3.2.8. PREPARATION OF CAPSULES

Table 3.7. Formula for the preparation of capsules

Ingredient	Master formula	Scaled quantities × 60
G-Rea powder	0.215g	12.9g
Lactose powder	0.290g	17.40g

3.2.8.1. Method

The calculation for the dose of the extract was based on the quantity of extract obtained from the drying of one dose of the herbal mixture on the market. 60ml (one dose) of the G-Rea herbal mixture was measured into a pre weighed porcelain dish and dried in a hot air oven at 45℃ to evaporate to dryness and weighed, it was further dried in the oven and weighed every 30 minutes until a constant weight was obtained. The weight of the dried extract was calculated by deducting the weight of empty porcelain dish from the weight of the porcelain and extract which gave the weight of extract in the one dose of the mixture. The experiment was done in triplicate. The size of capsule shell to be used was dependent on the weight of powder per dose of the drug and the tapped densities of the drug and excipients (Capehart, 2008).

The required weights of the extract and the excipient were calculated and weighed into a dry container and mixed well. Size ’0’ capsule shell of volume 0.68ml was used, the shells were opened and were fixed into a manual capsule filling machine. The powder mixture was poured onto the powder bed on the machine and slowly filled with the aid of a plastic spatula to fill the shells uniformly. Excess powder was removed and apparatus was tapped to ensure powder was well inside the shells (Bhatt and Aggrawal, 2007). The shells were covered with the caps and put in a well covered container and stored at room temperature for further evaluation.

3.2.8.2. EVALUATION OF CAPSULES

3.2.8.2.1. Uniformity of weight and disintegration tests

The procedures used for these tests are of the same specifications as used for pessaries as shown in subsections 3.2.6.4.2 and 3.2.6.4.3

3.2.8.2.2. Disintegration tests

The disintegration time of capsules was determined according to the procedure described in the British Pharmacopoeia (BP, 2007). A capsule was placed in each of the six tubes of the disintegration apparatus and the time taken for all capsules to completely disintegrate in distilled water maintained at 37 ± 2 ^{o}C were determined.

3.2.8.2.3. Dissolution tests on capsules

The method and apparatus as described in (3.2.7.5.3) was used and the sample introduced into the dissolution medium were the prepared capsules. The same time points and method of analysis were used.

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CHAPTER 4

RESULTS

4.1. Yield of extracts

Table 4.1. Yield of extracts

Extract	Weight of raw sample/g	Weight of extract/g	%yield
Ethanol (70%)	100	17.3	17.3
Water	100	10.9	10.9

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of powder}} \times 100\%$$

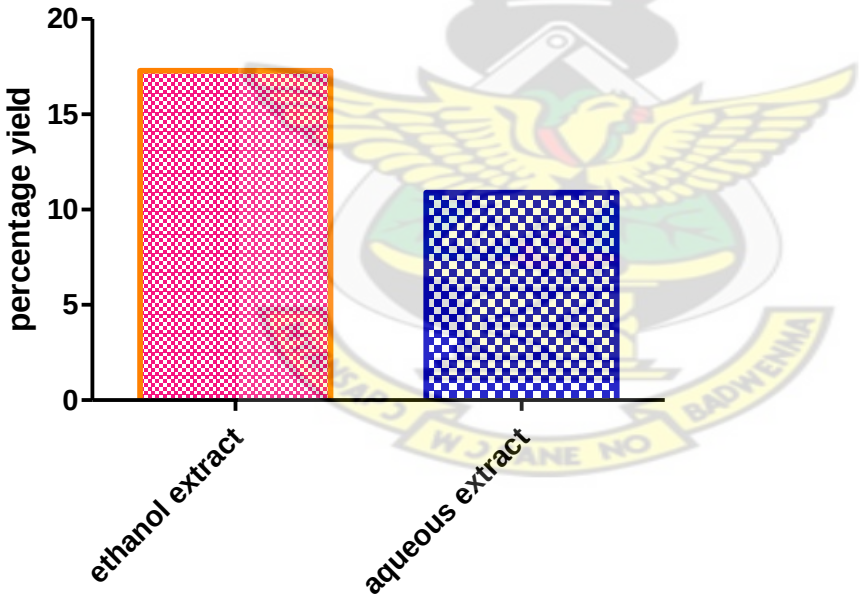

Figure 4.1: percentage yield of ethanol and aqueous extracts

4.2. Phytochemical screening of extracts

Table 4.2. Results of phytochemical screening

Bioactive compound	Raw powdered sample	Ethanolic extract	Aqueous extract	G-rea mixture sold on the market
Glycosides	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	-	-
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Steroids	-	-	-	-
Terpenoids	+	+	+	+
Anthraquinones	-	-	-	-

4.3. Physicochemical properties of extracts

Table 4.3. Physical appearance of extracts

Extract	Colour	Texture
Ethanol extract	Greenish brown	Powdery
Aqueous extract	Greenish brown	Sticky and lumped together

Table 4.4. Characteristics of decoctions.

	G-Rea (Osei Herbal)	G-Rea (with Benzoic acid)	G-Rea (without Benzoic acid	G-Rea (without aspartame)
pH	3.68	3.91	4.55	3.92
Colour	Yellowish brown	Greenish brown	Greenish brown	Greenish brown
Taste	Bitter	Slightly sweet	Slightly sweet	Bitter
Odour	Sharp	sharp	sharp	sharp
Clarity	No particles	No particles	No particles	No particles

Table 4.5. Moisture content determination of extracts

Extract	A	B	C	Mean±SEM
Ethanol extract	9.0%	9.2%	9.0%	9.07±0.12
Aqueous extract	10.0%	10.2%	10.5%	10.23±0.25

Table 4.6. Solubility testing of extracts

Dissolution medium	**Weight of extract(g) per ml of filterate**		
	Ethanolic extract	**Aqueous extract**	**Remarks**
70% ethanol	0.0042g	0.00288g	Soluble
Water	0.003g	0.004g	Soluble

Table 4.7. pH testing of extracts

Extract	**pH**
Ethanol extract	4.95
Aqueous extract	4.54

4.4. HPLC analysis of ethanolic extract

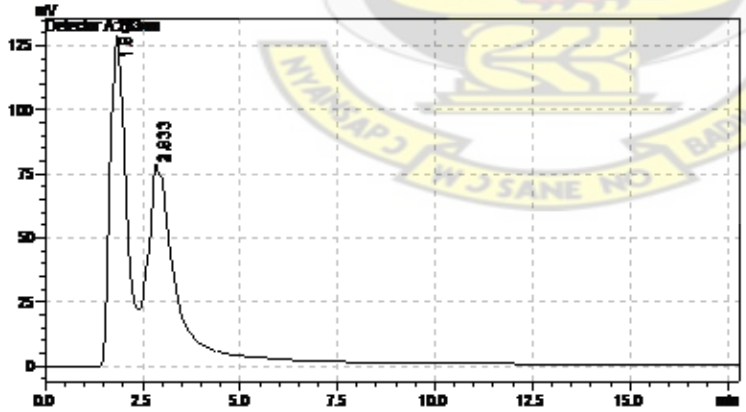

Figure 4.2. Chromatogram of 0.1g G-Rea extract in 100ml water .

Table 4.8. Flow properties of ethanolic extract

Initial volume (Vo/ml)	19	18	19	Mean values	Acceptable ranges
Tapped volume (V1/ml)	16	16	16.5		
Bulk density (g/ml)	0.53	0.55	0.53	0.54±0.01	0 -1 g/ml
Tapped density(g/ml)	0.625	0.625	0.606	0.62±0.01	0 -1 g/ml
Hausner's ratio	1.179	1.136	1.143	1.15 ±0.02	1 - 3
Carr's Index	15.2	12	12.54	13.25± 1.71	0 - 50
Angle of repose	35º	34º	33º	34º±1	0-50

Mass of extract : 10g

4.5. ANTIMICROBIAL PROPERTIES OF EXTRACTS

Table 4.9 Antimicrobial properties of ethanolic extract

	Mean zones of inhibitions (mm) + SEM				
Concentrations	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtillis*	*Candida albicans*
10%w/v	30.67±*1.15*	0	0	17.33±0.58	15.67±0.58
5%w/v	19.33±*1.15*	0	0	13.67±0.58	13.00±1.00
2.5%w/v	16.33±0.58	0	0	11.33± 0.58	0
1.25%w/v	11.33±0.58	0	0	0	0

Diameter of cork borer : 10mm

N value = 3

Table 4.10. Antimicrobial properties of aqueous extract

	Mean zones of inhibitions (mm) + SEM				
Concentrations	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtillis*	*Candida albicans*
10%w/v	26.67±0.58	0	0	17.33±0.58	12.67±0.56
5%w/v	21.67± *0.58*	0	0	11.33± *0.58*	0
2.5%w/v	16.33±0.58	0	0	0	0
1.25%w/v	0	0	0	0	0

Diameter of cork borer : 10mm, n = 3

SEM **:** standard error mean

4.6. Minimum inhibitory concentrations of the extracts against susceptible organisms

Table 4.11. MIC of extracts

	MIC (mg/ml)	
Organism	**Aqueous extract**	**Ethanolic extract**
Staphylococcus aureus	20	20
Bacillus subtillis	40	40
Candida albicans	60	50

Table 4.12. Antimicrobial activity of decoctions

	Mean zones of inhibitions (mm) + SEM				
Decoctions	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtillis*	*Candida albicans*
G-Rea (Osei Herbal)	12.66±0.58	0	0	11.67±0.58	13.66±0.58
G-rea (with benzoic acid and aspartame) (A)	0	0	0	0	0
G-rea (without benzoic acid) (B)	0	0	0	0	0
G-Rea (wit (hout aspartame) (C)	12±00	0	0	11.33±0.58	12.33±0.58

Diameter of cork borer : 10mm, n = 3

Table 4.13. PHYSICAL CHARACTERISTICS OF GELS

Product	pH	Colour	Taste	Odour	clarity
Gel with 3%w/v xanthan gum base	4.67	Dark brown	Sweet with bitter aftertaste	Orange/chocolate	No particles
Gel with 10%w/v starch as base	3.87	Dark brown	Sweet with bitter aftertaste	Orange/chocolate	No particles
Gel with 6% w/v CMC base	5.53	Dark brown	Sweet with bitter aftertaste	Orange/chocolate	No particles
Gel with 4%w/v CMC as base	5.30	Dark brown	Sweet with bitter aftertaste	Orange/chocolate	No particles
Gel with 2%w/v CMC as base	5.31	Dark brown	Sweet with bitter aftertaste	Orange/chocolate	No particles
Gel with 20%w/v HPMC as base	5.58	Dark brown	Sweet with bitter aftertaste	Orange/chocolate	No particles
20%w/v HPMC Gel with orange colour	4.91	Yellowish brown	Sweet with bitter aftertaste	Orange/chocolate	No particles
20%w/v HPMC Gel with white emulsion colour	4.94	Light brown	Sweet with sharp aftertaste	Orange/chocolate	No particles

CMC: Carboxymethyl cellulose

HPMC: Hydroxypropyl methylcellulose

Concentration of extract :10%w/w

Table 4.14. Antimicrobial activity of gels

	Mean zones of inhibitions (mm) + SEM		
Product	*Candida albicans*	*Bacillus subtillis*	*Staphylococcus aureus*
10%w/w gel with 3% xanthan gum base	18±0.56	0.00	22±00
10%w/w gel with starch as base	13±00	0.00	16±0.56
10%w/w gel with 6% CMC base	0.00	0.00	0.00
10%w/w gel with 4% CMC as base	12±00	0.00	14±0.56
8%w/w gel with 2%w/v CMC as base	0.00	0.00	0.00
10%w/w gel with 20% HPMC as base	17±0.56	12±0.56	27±0.56
10%w/w gel with white emulsion colour and 20%HPMC base	18±0.00	12±0.00	28±0.00
Daktarin oral gel	20 ±0.56	0.0	0.0

N = 3

HPMC: Hydroxypropyl methylcellulose

SEM: Standard error mean

Diameter of cork borer: 10mm

4.7. Analysis of Viscosity and pH of gels with time

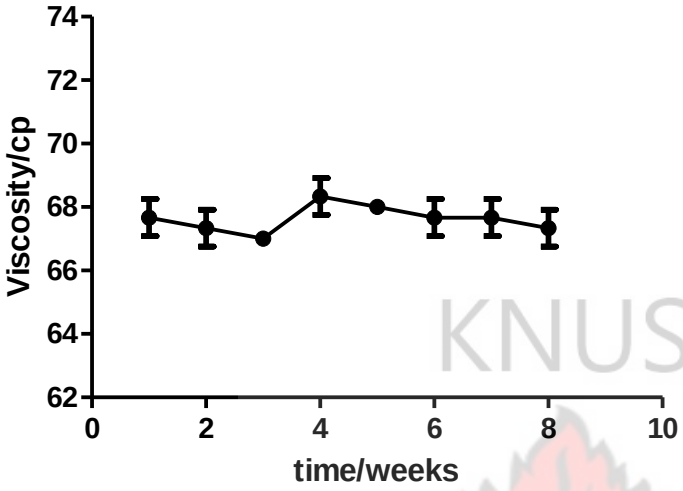

Figure 4.3. Graph of viscosity over time for Daktarin oral gel

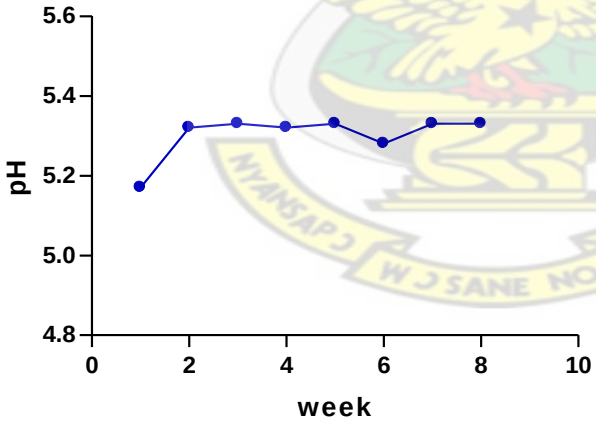

Figure 4.4. Graph of pH over time for Daktarin oral gel

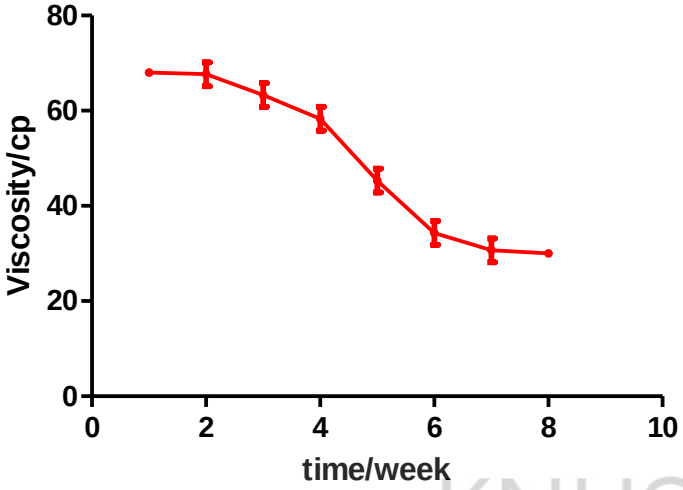

Figure 4.5. Graph of viscosity against time for 2%w/v CMC gel

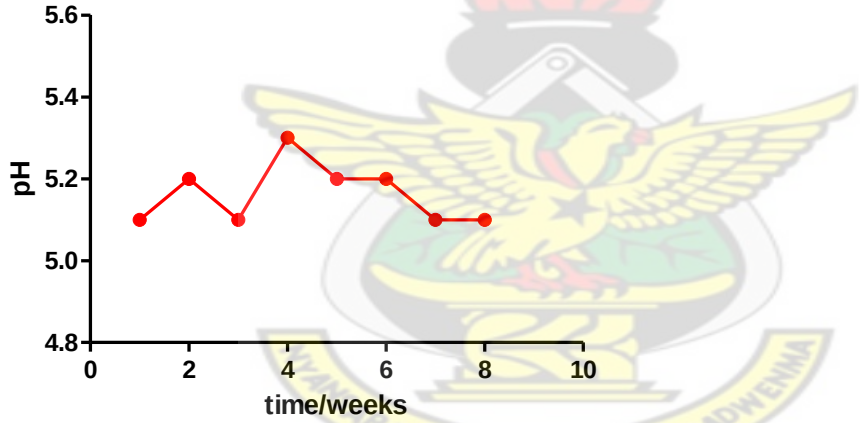

Figure 4.6. Graph of pH against time for 2%w/v CMC gel

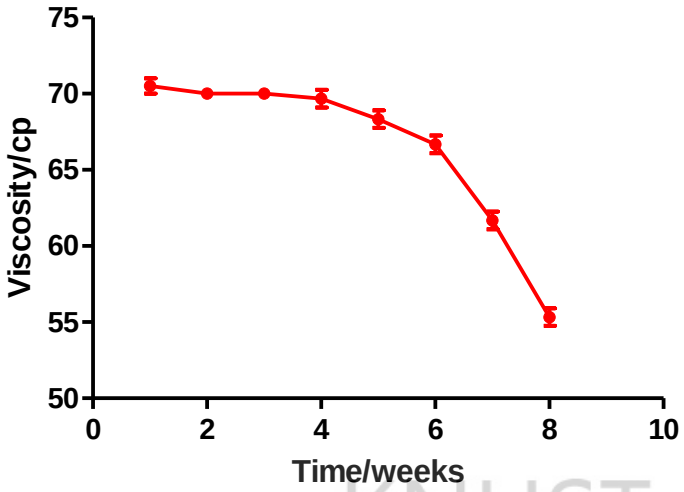

Figure 4.7. Graph of viscosity against time for 4%w/v CMC gel

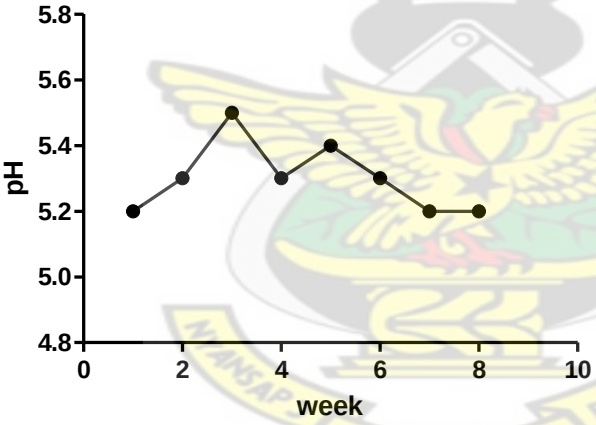

Figure 4.8. Graph of pH against time for 4%w/v CMC gel

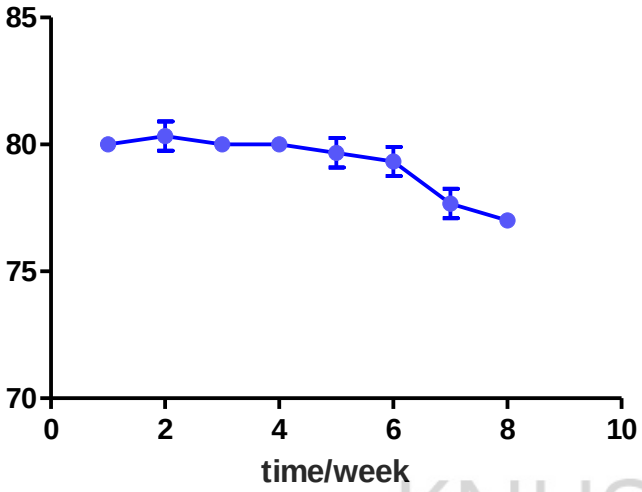

Figure 4.9. Graph of viscosity against time for 6%w/v CMC gel

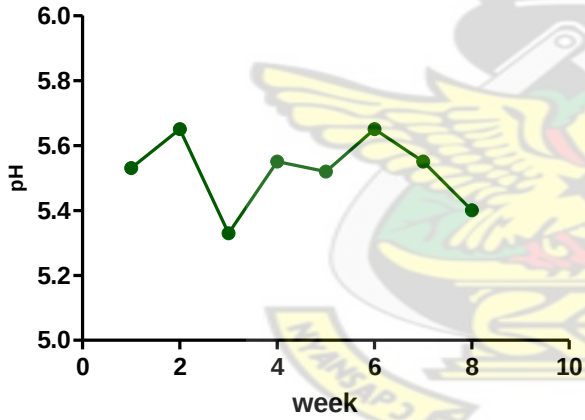

Figure 4.10. Graph of pH against time for 6%w/v CMC gel

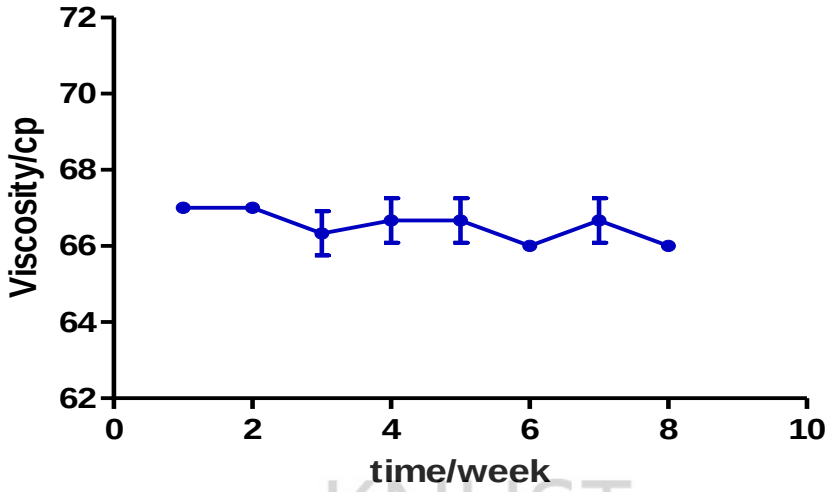

Figure 4.11. Graph of Viscosity against time for 20%w/v HPMC gel

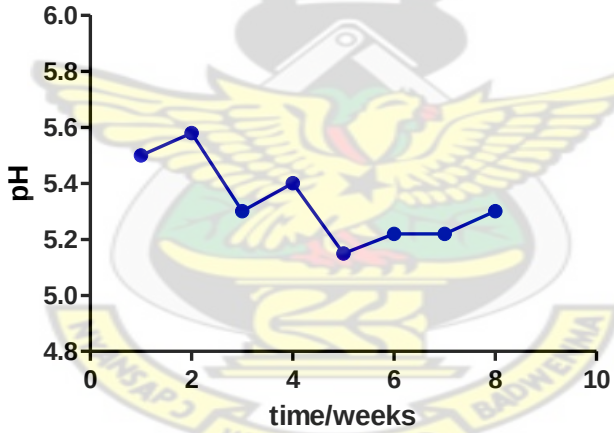

Figure 4.12. Graph of pH against time for 20%w/v HPMC gel

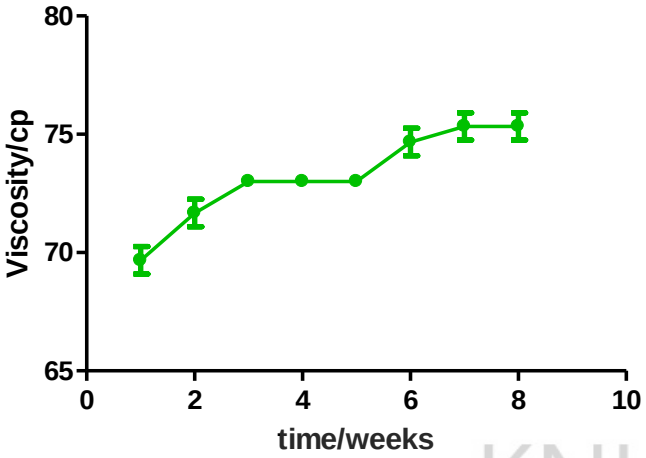

Figure 4.13. Graph of viscosity against time for 3%w/v xanthan gum gel

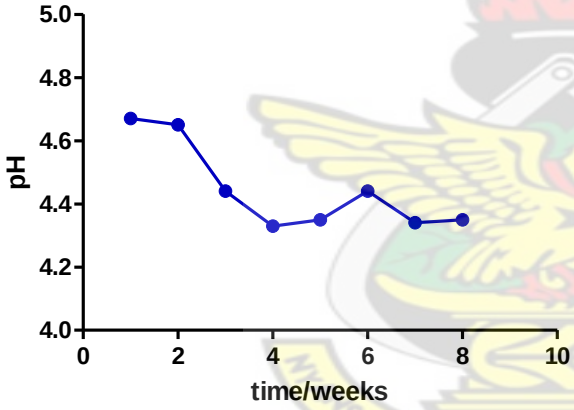

Figure 4.14. Graph of pH against time for 3%w/v xanthan gum gel

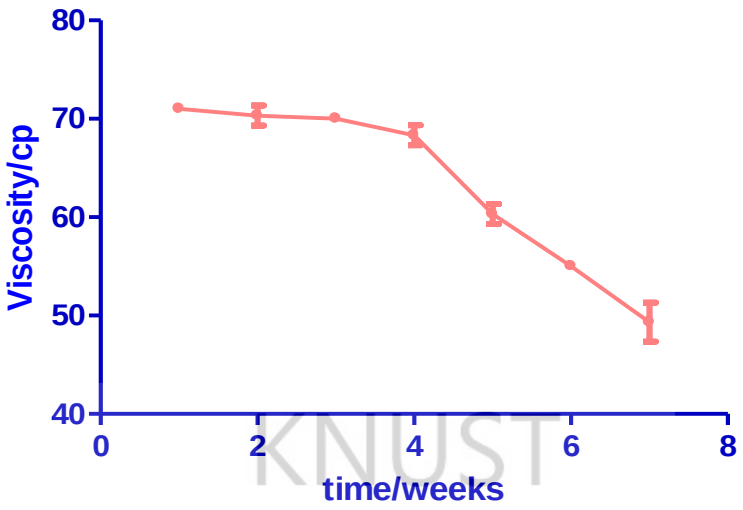

Figure 4.15. Graph of viscosity against time for 10%w/v pre-gelatinised potato starch gel

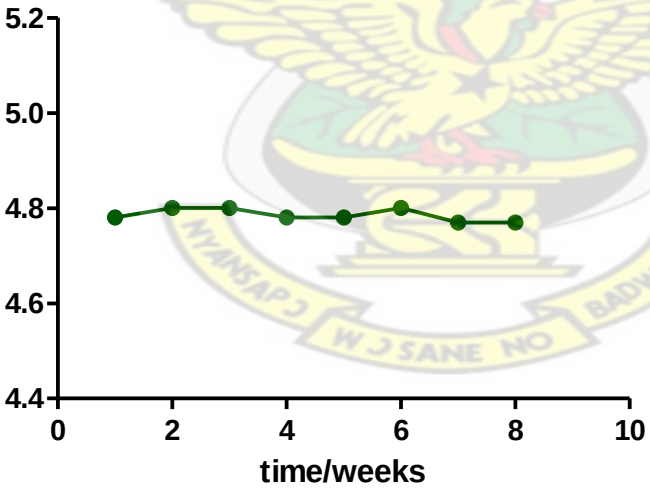

Figure 4.16. Graph of pH against time for 10%w/v pre-gelatinised potato starch gel.

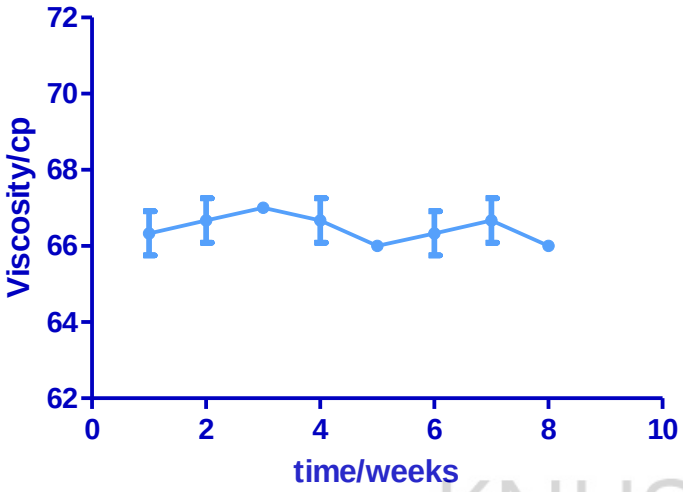

Figure 4.17. Graph of viscosity against time for 20%w/v HPMC gel with white emulsion colour.

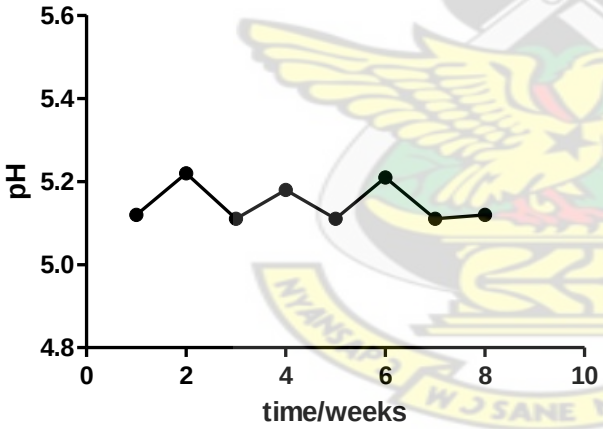

Figure 4.18. Graph of pH against time for 20%w/v HPMC gel with white emulsion colour

4.8. Comparism of the viscosities of the various gels with that of daktarin oral gel

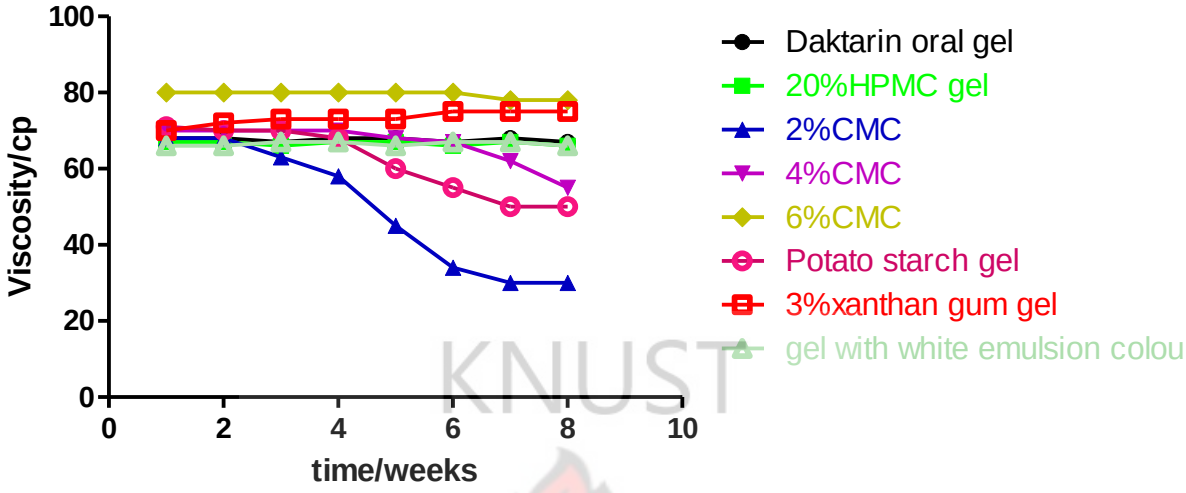

Figure 4.19. Graph comparing the viscosities of gels against time.

4.9.PESSARY PREPARATION

Table 4.15. Displacement value of plain pessaries

Product	Displacement value
Glycerogeatin pessaries	0.51
Theobroma oil + 10%w/w beeswax pessaries	1.25

4.9.1. Physical appearance of pessaries

The pessaries produced were brown in colour, had smooth surface and had no air bubbles when cut open. They did not melt at room temperature.

4.9.2. Quality control tests carried on pessaries

4.9.2.1. Uniformity of weight test

CALCULATION

The percentage deviations of the pessaries from the mean were calculated using:

Percentage deviation = $\frac{A-B}{B} \times 100$

Where, A= Individual weight of pessaries, B = Average weight of 20 pessaries.

Table 4.16. Uniformity of weight for G-Rea herbal pessaries with glycerogelatin base

Pessary no.	Pessary weight (Ag)	(A-B)g	Percentage deviation$(A-B)/B \times 100$
1	2.370	0.0349	1.495
2	2.351	0.0159	0.681
3	2.379	0.0439	1.880
4	2.325	-0.0101	-0.433
5	2.319	-0.0161	-0.689
6	2.299	-0.0361	-1.546
7	2.330	-0.0051	-0.218
8	2.332	-0.0031	-0.133
9	2.328	-0.0071	-0.304
10	2.335	-0.0001	-0.004
11	2.311	-0.0241	-1.032
12	2.351	0.0159	0.681
13	2.301	-0.0341	-1.460
14	2.330	-0.0051	-0.218
15	2.301	-0.0341	-1.460
16	2.355	0.0199	0.852
17	2.365	0.0299	1.280
18	2.320	-0.0151	-0.647
19	2.343	0.0079	0.338
20	2.356	0.0209	0.895
	Total Weight = 46.702		
	Average weight = 2.3351		
	Standard deviation = ±0.023		

Table 4.17. Uniformity of weight test for G-Rea herbal pessaries with theobroma oil + 10% beeswax base

Pessary no.	weight of pessary (A)g	Deviation (A- B)g	Percentage deviation $\frac{A-B}{B} \times 100$
1	1.871	-0.0735	- 3.78
2	1.882	-0.0625	- 3.21
3	2.000	0.0555	2.85
4	1.884	-0.0605	-3.11
5	2.005	0.0605	3.11
6	1.979	0.0345	1.77
7	2.008	0.0635	3.26
8	1.881	-0.0635	-3.26
9	1.906	-0.0385	-1.98
10	1.979	0.0345	1.77
11	1.969	0.0245	1.26
12	1.961	0.0165	0.85
13	2.002	0.0575	2.96
14	1.989	0.0445	2.29
15	1.923	-0.0215	- 1.11
16	1.923	-0.0215	- 1.11
17	1.941	-0.0035	- 0.18
18	1.918	-0.0265	-1.36
19	1.923	-0.0215	-1.11
20	1.941	-0.0035	-0.18

Total Weight = 38.890

Average weight = 1.9445

Standard deviation = ±0.047

Table 4.18. Disintegration test results on pessaries

	Disintegration time/minutes			
TEST	1	2	3	mean± SEM
Pessary with glycerogelatin base	19	18.5	20	19.17±0.76
Pessary with theobroma +10%w/w beeswax base	8.5	9	9	8.83±0.29

Table 4.19. Antifungal activity of pessary with glycerogelatin base against *Candida albicans*

Test	1	2	3	mean±SEM
Zone of inhibition (mm)	21	21	20	20.67±0.58

Diameter of cork borer: 10mm

4.9.3. DISSOLUTION PROFILE G-REA HERBAL PESSARIES FORMULATED WITH DIFFERENT DIFFERENT BASES

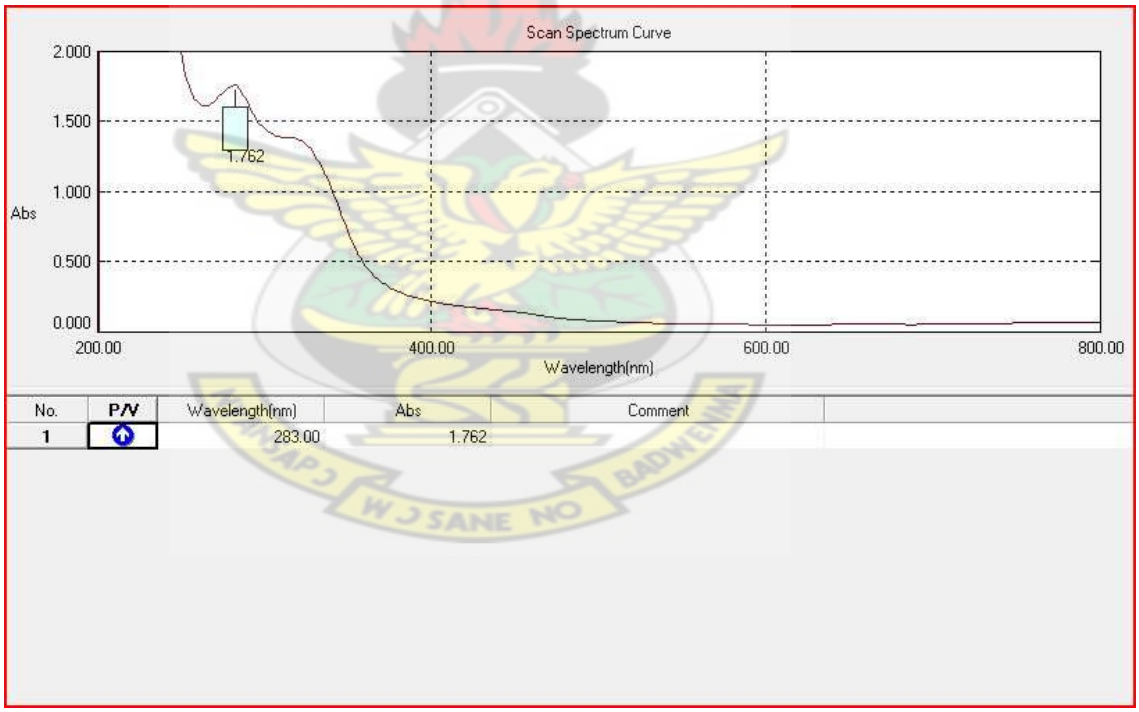

Figure 4.20. UV Spectrum graph of ethanol extract of G-Rea powder

Blank used: 0.1M HCl

4.9.4. Calibration curve for G-Rea herbal extract in 0.1MHCl at a wavelength of 283nm

Table 4.20. Absorbance of G-Rea herbal extract in 0.1MHCl

Concentration (%w/v)	Absorbance
0.0250	0.690
0.0150	0.564
0.0125	0.491
0.0100	0.440
0.0075	0.429
0.0050	0.392
0.0025	0.319

1.

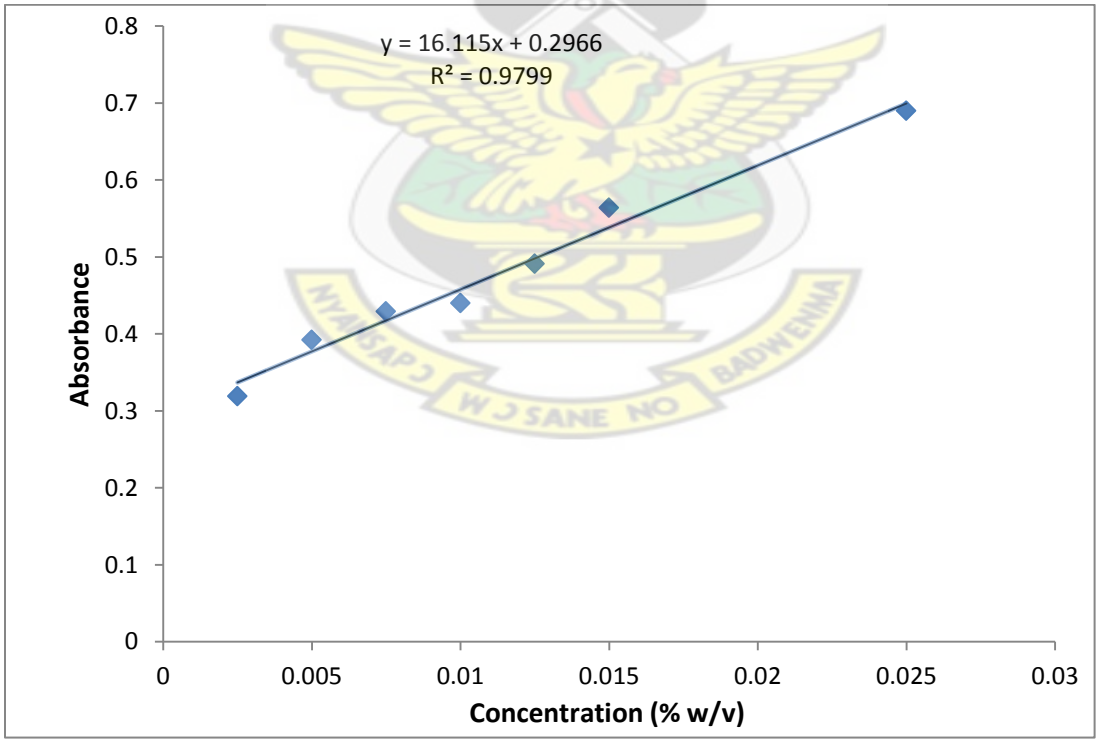

Figure 4.21. Calibration curve for G-Rea Herbal extract in 0.1M HCl

4.9.5. DRUG RELEASE PROFILE OF PESSARIES

4.9.5.1. Calculations of % release pessaries with glycerogelatin base

Weight per dose of G-Rea extract = 240mg (0.24g)

Volume of dissolution medium = 900ml

Concentration of solution if all 240mg dissolves = $\frac{0.24\times100}{900}$ =0.0267%w/v

In order to obtain accurate absorbance readings,1ml of the filterate was diluted to 5ml in a volumetric flask.

Equation of calibration curve is y = 16.115x + 0.2966

X = concentration

Y = average absorbance at a specific time

At 5 minutes for pessary, y = 0.323

Therefore x at 5 minutes = $\frac{0.323-0.2966}{16.115}$ = 0.00164%w/v

Therefore 100ml of solution = 0.00164g of extract

5ml = $\frac{5\times0.00164}{100}$ = 0.000082g

But 1ml of extract = 0.000082g

900ml = 900× 0.000082g =0.0738g

Weight of extract released = 0.0738g

% Release = $\frac{\text{weight of extract released}}{\text{weight of extract used}} \times 100$

Hence % Release = $\frac{0.0738}{0.24} \times 100 = 30.75\%$

At 15 minutes

y = 0.340

Therefore;

x at 15 minutes = $\frac{0.340-0.2966}{16.115}$ = 0.0027%w/v

Therefore 100ml of solution = 0.0027g

5ml = $\frac{5\times0.0027}{100}$ = 0.000135g

But 1ml = 0.000135g

Hence 900ml = 0.000135× 900 = 0.1215g

Weight of extract released = 0.1215g

Weight of extract in 10ml aliquot pipette at 5 minutes;

900ml = 0.0738g

Therefore 10ml = $\frac{10}{900}$ × 0.0738 = 0.00082g

Hence total weight of extract released at 15 munites = 0.1215+ 0.00082

= 0.1223g

% Release = $\frac{\text{weight of extract released}}{\text{weight of extract used}} \times 100$

Hence % release = $\frac{0.1223}{0.24}$ × 100 = 50.83%

The calculations were repeated for other percentages released at various times and for different products.

Table 4.21. Drug release profile for pessaries with glycerogelatin base

Time/minutes	Mean absorbance	Percentage release
5	0.323	30.75
15	0.340	50.83
30	0.350	63.04
45	0.365	81.18
60	0.374	92.53

Table 4.22. Drug release profile for pessaries with Theobroma + 10%w/w beeswax base

Time/minutes	Mean absorbance	Percentage release
5	0.308	16.09
15	0.310	18.87
30	0.313	23.29
45	0.315	26.38
60	0.320	33.60

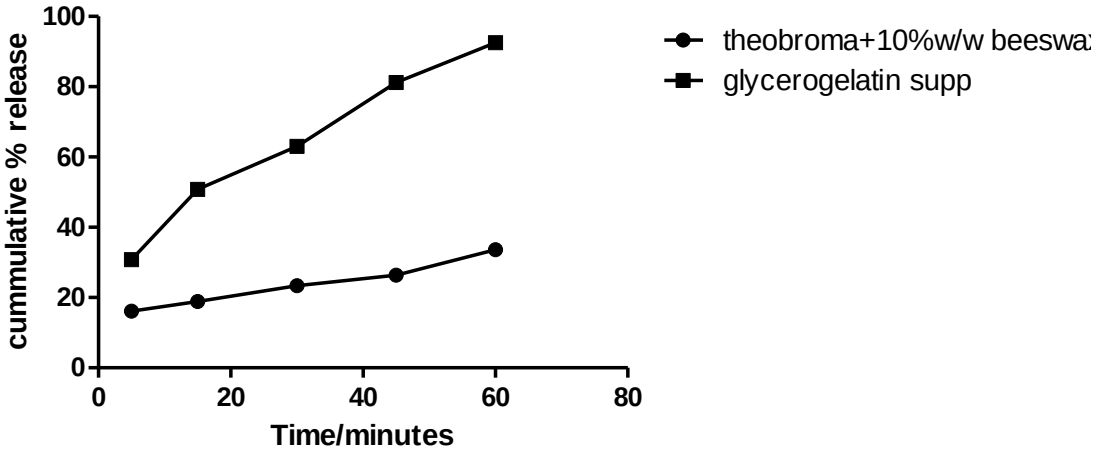

Figure 4.22. Graph showing the dissolution profile of pessaries

4.10. CAPSULE FORMULATION

Table 4.23. Calculation of weight per dose of G-Rea herbal mixture

Dish	A	B	C
Weight of dish + extract(g)	89.97	29.08	43.70
Weight of empty dish(g)	89.54	28.64	43.28
Weight of extract (g)	0.43	0.44	0.42

$$\text{Mean weight} = \frac{0.43+0.44+0.42}{3}$$

Mean weight = 0.430g ± 0.01

Table 4.24. Uniformity of weight test for G-Rea capsules

Capsule no.	Capsule weight(A)g	Deviation (A-B)	Percentage deviation[(A-B)/B]×100
1	0.61	0.012	1.98
2	0.584	-0.014	-2.37
3	0.601	0.003	0.48
4	0.602	0.004	0.64
5	0.591	-0.007	-1.20
6	0.599	0.001	0.14
7	0.611	0.013	2.15
8	0.601	0.003	0.48
9	0.608	0.010	1.65
10	0.592	-0.006	-1.03
11	0.61	0.012	1.98
12	0.591	-0.007	-1.20
13	0.588	-0.010	-1.70
14	0.600	0.002	0.31
15	0.582	-0.016	-2.70
16	0.601	0.003	0.48
17	0.581	-0.017	-2.87
18	0.601	0.003	0.48
19	0.600	0.002	0.31
20	0.610	0.012	1.98

Total Weight = 11.963

Average Weight = 0.598

Standard Deviation = $\pm$0.010

Table 4.25. Disintegration test results

Disintergration time/ minutes				
TEST	1	2	3	Mean ±SEM
Time (minutes)	5	5	5.5	5.17±0.29

4.10.1. Dissolutiuon test results

Calculations as shown in 4.7.5.1 were used in calculating for the percentage release of the capsules.

Table 4.26. Drug release profile for G-Rea Herbal Capsules

Time/minutes	Mean absorbance	Percentage release
5	0.346	32.08
15	0.368	46.72
30	0.380	55.03
45	0.410	75.13
60	0.416	79.83

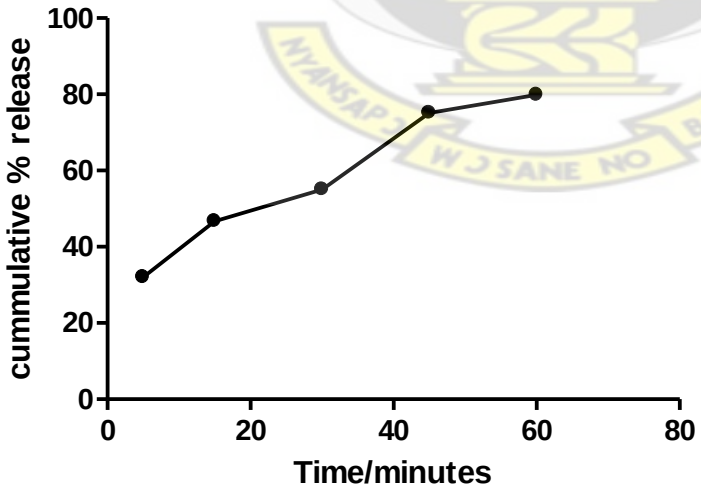

Figure 4.23. Graph showing the release profile of G-Rea capsules

CHAPTER FIVE

DISCUSSION

5.1.Percentage yield

The percentage yield obtained for the extraction using 70% ethanol was 17.3% whiles the aqueous extraction process yielded 10.9% as shown in table 4.1 and figure 4.1. This could be reasoned to a higher solubility of the components in 70% ethanol. Therefore using 70% ethanol in the extraction gives a better yield compared to that of water.

5.2. Extraction, phytochemical and antimicrobial tests results

The study on both ethanol and aqueous extracts of G-Rea herbal powder revealed that both extracts had antimicrobial activities against *Staphylococcus aureus*, *Bacillus subtillis* and *Candida albicans.* The phytochemical screening of the powdered plant sample and the extracts revealed the presence of various phytochemicals which include; alkaloids, tannins, flavonoids and terpenoids, glycosides and saponins, however the raw powder and the ethanol extract contained flavonoids whiles the aqueous extract and the herbal mixture sold on the market did not as shown in Table 4.2. Tannins and terpenoids are known for their antimicrobial activities (Chung, 1998), therefore the antimicrobial activity of the extracts may be attributed to the presence of these phytochemicals.

HPLC analysis is used in identification and standardization of herbal medicines. It can also be used in quantification in analytical chemistry. HPLC analysis of the ethanolic extract showed two major peaks (figure 4.2), indicating the presence of two major components which can further be isolated and analysed.

The ethanolic extract of the powder was selected for the preparation of the products because it gave a higher extractive value of 17.3% compared to that of the aqueous extract which gave 10.9%. The aqueous extract had very bad flow properties as they were sticky, lumped together and could not even be powdered. Also the ethanol extract had a higher antimicrobial activity against the susceptible organisms and had a lower moisture content of 9.02 compared to that of the aqueous extract (table 4.5). The Minimum Inhibitory Concentrations (MIC) values obtained for the ethanol extract was 50mg/ml for *Candida albicans,* 20mg/ml for *Staphylococcus aureus* and 40mg/ml for *B.subtillis* , whiles the aqueous extract produced 60mg/ml for *Candida albicans,* 20mg/ml for *Staph.aureus* and 40mg/ml for *B.subtillis* as seen in table 4.11. The ethanolic extract gave a lower MIC for *Candida albicans* compared to that of the aqueous extract and because the products to be produced were mainly for antifungal activity, the selection of the ethanol extract was justified.

Solubility: A substance is said to be soluble if 0.1g of it dissolves in 100ml of solvent (Rogers *et al*., 1987) From the results shown in table 4.6, 0.0042g and 0.003g of the ethanolic extract dissolved in 1ml of 70% ethanol and water respectively which means 0.42g and 0.3g of the extract would dissolve in 100ml of 70% ethanol and water respectively. This indicates solubility in both solvents.

The aqueous extract also showed solubility in water and 70% ethanol as shown in table 4.6. Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for achieving required pharmacological response. Poorly water soluble drugs often require high doses in order to reach therapeutic plasma concentrations after oral administration. Therefore solubility of the extracts in water is good for the achievement of desired therapeutic response.

5.3. Decoctions

From table 4.12, two of the decoctions (products A and B) did not show antimicrobial activity against the test organisms except the decoction without aspartame (product C) which showed activity against *Staph.aureus, B.subtillis* and *Candida*. However the non inclusion of a sweetener gave a product which was bitter and very difficult to take. This shows that the quantity of aspartame added to the decoction could have an effect on the activity of the decoction. However, from table 4.13, G-Rea herbal mixture from Osei herbal centre showed higher antimicrobial activity against susceptible organisms, comparing the antimicrobial activity of product C to that of G-Rea herbal mixture from Osei herbal, it can be observed that product C had slightly lower activity against susceptible organisms. The failure of decoction A to show activity could not be explained since it was prepared using the formula and method of preparation as given by Osei Herbal centre.

5.4. Physical properties of extracts

The extracts had a greenish-brown colour which is an indication of the probable use of leaves, stem and/or roots of the individual plants in the powdered sample. The pH was found to be 4.9 for the ethanol extract and 4.54 for the aqueous extract. This makes the extracts suitable for use in the preparation of vaginal pessaries and oral products. The normal vaginal pH is between 3.8 to 4.5, which is required for a healthy vaginal function and protection purposes. A pH above 5 promotes the growth of pathogenic organisms in the vagina (Jahić *et al*., 2006). Formulating pessaries with extracts whose pH is within the normal pH range of the vagina can lead to products which will achieve its purpose and also not disturb the normal microbial flora of the vagina. Also the drug's pH would not be altered to affect its potency.

Moisture content : The results of moisture content as shown in table 4.5 indicates that, the ethanol extract had a moisture content of 9.07% while the aqueous extract had 10.23%. According to the European pharmacopoeia (2007), weight loss on drying should not exceed 10% when dried for 2 hours. Though the ethanolic extract had values within the normal range, the aqueous extract did not. Moisture content gives an indication of the stability of extract on storage, high moisture content of an extract could lead to microbial contamination and chemical reaction on storage. Lower moisture content is always desired in powders. The results show that, the powders would have to be stored in an air tight container so as not to absorb excessive moisture.

5.4.1.Flow properties of ethanolic extract

The knowledge of the flow properties of powders is of critical significance in operations such as blending, tablet compression, capsule filling and in scale-up operations (Sarraguça *et al.*, 2010). The ethanol extract had good flow properties as was shown by the results in table 4.8. With a bulk density of 0.54, tapped density of 0.62, Hausner's ratio of 1.15, Carr' index of 13.5 and angle of repose of 34º it can be said all the parameters fell within the acceptable range as indicated in the USP (2007).

5.5. Oral gels

5.5.1. Physical appearance

The developed herbal gels were dark brown in colour, translucent in appearance and showed good homogeneity with consistency as shown in table 4.13. They had sweet taste but with bitter after taste. The sweet taste could be due to the addition of a sweetener (i.e. aspartame and glycerol) which masked the bitter taste of the extract, while the bitter after taste is due to the strong bitter taste of the extract. Orange and cocoa flavours were added to the formulations to give them pleasant oduor in order to ease their administration. Gels which had orange colour and white emulsion colour added respectively had yellowish brown and light brown colours. Physical appearance of products is very important in formulations. A pleasant physical appearance can enhance the adherence and compliance to medications (Oliver, 2011). White emulsion colour improved the appearance of the gel and made it more presentable compared to the other gels which were dark brown in colour as shown in table 4.13.

5.5.2. Antimicrobial activity and stability studies of gels

Stability studies are conducted at all phases of drug development cycle for different purposes with the ultimate goal of having a stable product on the market. During dosage form development, stability studies are conducted to support the formulation development and safety and efficacy ((Du *et al*., 2011). Antimicrobial studies are also conducted to test for the efficacy of antimicrobial property at specific concentrations. During the period of study, the gel with 2% CMC gelling agent and 8%w/w of extract (product I) showed no noticeable antimicrobial activity against susceptible organisms (as shown in table 4.14) and no change in pH as indicated in figure 4.6. The viscosity of the product remained consistent for two weeks after which it began to decline gradually from 68cp to 30cp by the 8th week. Comparing the viscosity of product I, to that of Daktarin oral gel (figure 4.3.) it can be said that product 'I'

does not have a consistent viscosity because both were stored under the same conditions. This showed that at lower concentration of the extract in the gel, the product was not able to release the extract for antimicrobial activity, also the 2%w/v CMC gelling agent product did not give a product with consistent viscosity.

All the other gels produced had antibacterial and antifungal activities when the concentration of the extract was increased to 10%w/w, they had consistent pH throughout the period of study. In viscosity testing, gel with xanthan gum base had its viscosity increasing gradually from 70cp to 75cp on the 8th week (figure 4.13). Gels should have consistent viscosity and as such, using xanthan gum will not be suitable in this formulation.

With 4%w/v CMC as a gelling agent and 10%w/w of extract, the resultant product showed antimicrobial activity (table 4.14), however the viscosity of the product remained at 70cp for four weeks after which it began to fall gradually to 55cp by the 8th week as shown in figure 4.7. This shows that using 4%w/v CMC as a gelling agent in the formulation could not give a stable product. Gel with 6%w/v CMC as base had stable viscosity for 6 weeks after which it began to decline as shown in figure 4.9. The viscosity of the product was 80cp which is high compared to that of Daktarin oral gel which was between 67cp and 68cp. This formulation did not show any antimicrobial activity, probably due to its high viscosity. High viscosity of gels negatively affects the release of active ingredients from gels (Prakash *et al*., 2010).

Using potato starch as a gelling agent and 10%w/w extract gave a product which had antimicrobial activity against susceptible organisms as shown in table 4.14, but its viscosity dropped gradually from 71cp on the first week to 55cp by the 8th week as indicated in figure 4.15. This showed that using potato starch as a gelling agent was not suitable for the formulation of G-Rea gel. In the preparation using 3%w/v xanthan gum as the gelling agent,

the product had an initial viscosity of 70cp but rose gradually to 75cp by the 8th week as shown in figure 4.13, this shows a product that could not sustain a constant viscosity and as such not stable on storage. The pH did not fluctuate much (figure 4.14), the product showed antimicrobial activity against susceptible organisms (table 4.14). Comparative study of the results shows that, the use of xanthan gum may not be suitable as a gelling agent in this production.

The use of 20%w/v hydroxypropyl methylcellulose as a gelling agent gave a product which had a consistent viscosity of between 66cp to 67cp for the 8 week period of study as shown in figure 4.11. It also showed antimicrobial activity against *Candida albicans* (table 4.14) , the pH of 5.2 to 5.58 (figure 4.12) obtained for the period of study within the acceptable range of 5.5-7.5 as stated by the BP. Comparing these results to that of Daktarin oral gel, which had a viscosity between 67cp to 68cp and a pH between 5.28 to 5.33, it showed that using 20%w/v hydroxypropyl methyl cellulose as a gelling agent gives a product which has similar characteristics as Daktarin oral gel. As such it can be used as a suitable gelling agent in the preparation of G-Rea oral gel.

Addition of white emulsion colour in the formulation of hydroxypropyl methyl cellulose gel also gave a product with consistent viscosity and which was close to that of the reference drug (figure 4.17). Again there was no pH fluctuation of the product as shown in figure 4.18. The product also had antimicrobial activity against susceptible organisms as shown in table 4.14. The addition of the colour also improved the appearance of the product. This showed that this colour can be added in the formulation to improve the colour of the gel.

Addition of orange colour to the 20%w/v HPMC gel gave a product with improved colour but was staining the mouth, which was undesirable, as such orange colour cannot be recommended for use in the production of the oral gel.

Pharmaceutical stability is a key determinant of product formulation success, therapeutic efficacy and toxicity of medications. The objective of leaving the product on the bench for the eight week period and studying viscosities and pH changes as a function of time was to evaluate the stability of these gels. Stability testing is to provide evidence as to how the quality of a drug product varies as a function of time and storage conditions such as temperature, humidity, and light, which allows determination of shelf life for a drug product. Stability testing provides evidence that the quality of a drug substance or drug product under the influence of various environmental factors changes with time. The information obtained from stability studies can subsequently be used to provide guidelines on handling and storage, and provide information to guide formulation stabilization strategies (Du *et al*., 2011).

5.6. PESSARIES

5.6.1.Physical appearance

Pessaries produced were smooth to touch, oval shaped and had no air bubbles when cut open. They did not melt at room temperature and disintergrated at 37±0.5℃. 10%w/w beeswax was added to the theobroma base as a hardening agent because using theobroma base alone produced a pessary that had a very low melting point and sticky to touch.

5.6.2. Disintegration test

Disintegration test is one of quality control tests for pessaries. The results of the disintegration tests of the pessaries as indicated in table 4.18, shows that the pessaries had disintegration times that were within the stipulated range. The glycerogelatin pessaries had a disintegration time of 19.17 minutes whiles theobroma based pessaries had a disintegration time of 8.83

minutes. According to the BP 2007, disintegration should occur in not more than 30 minutes for fat based suppositories and not more than 60 minutes for water soluble suppositories. Hence the pessaries disintegrated within the acceptable range. Pessaries having low disintegration time have the advantage of having quicker absorption of the drug and having quicker onset of action, however, it can lead to the leakage of the drug from the vagina leading to reduction of therapeutic effectiveness. With relatively high disintegration time the drug has the advantage of staying in the site of action for a long time, prolonging its activity where local action is required. However it leads to delay in onset of therapeutic action especially when systemic action is required.

5.6.3. Uniformity of weight test

The uniformity of weight test gives an indication of the degree of uniformity of the amount of drug substance among dosage units. Standard deviation gives an indication of how the weights of the individual pessaries are scattered about the average weight. By British Pharmacopoeia standards for pessaries, the permitted percentage deviation for a pessary of any batch of any weight is 5%. Not more than two of the individual pessaries should deviate from the average weight by more than the permitted percentage deviation and none should deviate by twice the permitted deviation. From the results in table 4.16 and table 4.17, none of the pessaries deviated by 5%. The highest deviation for the pessaries with glycerogelatin base was 1.880% whiles that for theobroma base pessaries was -3.78%. This indicates uniform weight of the pessaries and hence uniform distribution of the extract between the pessaries. Considering the standard deviations values, pessaries with glycerogelatin base had better uniformity of weight than pessaries with theobroma base.

5.6.4. Dissolution tests

Pharmaceutically, dissolution is defined as the rate of mass transfer from a solid surface into the dissolution medium or solvent under standardized conditions of liquid/solid interface, temperature and solvent composition. The basic step in drug dissolution is the reaction of the solid drug with the fluid and/or the components of the dissolution medium. This reaction takes place at the solid—liquid interface and therefore dissolution kinetics are dependent on three factors, namely the flow rate of the dissolution medium toward the solid—liquid interface, the reaction rate at the interface, and the molecular diffusion of the dissolved drug molecules from the interface toward the bulk solution (Singhvi and Singh, 2011). Dissolution of drugs is very important in the absorption of drugs and subsequent pharmacological activity of the drug.

The UV spectra of ethanol extract of G-Rea powder showed clear markers at maximum wavelengths of absorption of 283nm as shown in figure 4.20.

From the calibration curve, Fig.4.21, it was observed that the coefficient of determination (R^2) value was 0.9799 which is indicative of good linearity of the calibration curve and made the subsequent determinations from the calibration curve valid.

According to the BP, for non modified release dosage forms, not less than 70% of the drug should be released by the 45th minute of being in the dissolution medium. From the results (table 4.21), pessaries with glycerogelatin base had 81.18% release by the 45th minute, while the pessaries with theobroma+ 10%w/w beeswax base had 26.38% release at the 45th munite (table 4.22). This indicates a good release profile for the pessary with glycerogelatin base. The low release profile of the pessary with theobroma +10%w/w base could be due to inability of the base to release the drug for dissolution. This could be due to the interaction between the extract and the base or hydrophobic interaction of this base in the aqueous environment

(dissolution medium). Low release affects the availability of active ingredients at the site of action for pharmacological activity. From the results, using theobroma +10%w/w beeswax base may not be suitable in the formulation of G-Rea herbal pessaries.

5.6.5. Antimicrobial tests on glycerogelatin base pessaries

Pessary with glycerogelatin base showed antifungal activity against *Candida albicans.* From the results as shown in table 4.19, it gave a zone of inhibition of 20.67 ± 0.58 mm, which shows that the pessaries can be used in the treatment of *Candida albicans* infections for its local effect.

5.7. CAPSULES

5.7.1.Formulation

Capsule shell size '0' was used in the formulation because of availability at the time of formulation. From the calculation of weight of extract per dose of the herbal mixture, it was found that one dose (60ml) of the product contained about 0.43g of dry extract. This method was used in determining the dose because the herbal mixture is already being used to treat patients and all the phytoconstituents present in the herbal mixture were also found present in the ethanolic extract except flavonoids as shown in table 4.3. Each capsule was filled with 215mg of the extract because from the tapped density of the extract, one size '0' of volume 0.68ml shell could not contain the 430mg of the extract, so the extract was divided into two, this means that a patient would have to take two capsules as a single dose. According to the manufacturers of the herbal mixture a patient should take 60ml (430mg) twice in a day which means one would have to take two capsules twice in a day. Because 215mg of the extract was not enough to fill the entire volume of the capsule shell, enough excipient was mixed with the extract to fill the capsule. Lactose was the excipient selected because of its wide range of compatibility, excellent flow properties and its wide use as a diluent. The quantity of lactose

used was based on the tapped density of the dried lactose powder and the volume of the empty space of capsule shell as shown in appendix C.

5.7.2. Uniformity of weight test for capsules formulated

From the results of the uniformity of weight test (table 4.24), it was found out that all capsules had weights well within the acceptable weight range. According to the BP 2007, for capsules which are 300mg or more, not more than 2 capsules should deviate from the average weight by 7.5% and none should deviate by twice that. The percentage deviation obtained from the results shows that none of the capsules deviated by 7.5% as the highest deviation recorded was 2.87%. Good flow properties enhance uniform filling of capsules. Hence the uniformity of weight of the formulated capsules was good and passed the BP 2007 specification of uniformity of weight test.

5.7.3. Disintegration test

Disintegration tests is a quality control test that shows the time it takes for a solid dosage form to disintegrate in an aqueous medium under prescribed experimental conditions. According to the BP the disintegration time of hard gelatin capsules should not be more than 30 minutes. According to the results obtained from the tests and shown in table 4.25, the disintegration time was 5.17 ± 0.29 minutes. Disintegration of capsules causes the capsule content to de-aggregate into multiparticulate system for dissolution. This result indicated that the capsules prepared disintegrated within the acceptable time range and as such will release the drug on time for dissolution to take place. Hence the formulated G-Rea capsules passed the BP 2007 specification for disintegration test.

5.7.4. Dissolution tests

The dissolution of drugs is very important in the absorption and subsequent activity of the drug. Dissolution tests performed on solid dosage forms to measure the drug release from the drug product as a test for product quality assurance and to determine the compliance with the dissolution requirements when stated in the individual monograph (Gupta *et al*., 2009). The BP (2007) states that, for non-modified release dosage forms, not less than 70% of the drug must be released by the 45th minute. From the results as shown on table 4.26, the percentage release by the 45th minute was 75.13, indicating a good release. This means that drug can dissolve in physiological solution to make available active ingredients for absorption and subsequent pharmacological activity. It also showed that the drug is suitable for use.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

Extraction of G-Rea powder using ethanol gave a higher percentage yield . Both ethanol and water extracts contained the same bioactive compounds including tannins, alkaloids, glycosides etc. except flavonoids which was not present in the aqueous extract. Both ethanol and aqueous extracts had antimicrobial activity against *Candida albicans, Staphyloccocus aureus* and *Bacillus subtillis*. HPLC analysis of the ethanolic extract showed two peaks, indicating the presence of two major components.

Ethanolic extract of the powder had good flow properties.

The extracts can be formulated into gels, capsules and pessaries.

The use of 20%w/v Hydroxypropyl methycellulose as a gelling agent and 10%w/w of the extract gave a gel formulation that is stable and had antimicrobial activity.

Capsules had good release profile.

The dissolution profile of pessaries depended upon the base used in the formulation. Using glycerogelation base gave pessaries with good release profile .

The quantity of aspartame used in the formulation of the herbal mixtures had an effect on the activity of the finished product.

6.2. Recommendations

Further studied could be conducted to

(i) Identify the various active constituents of the powder and

(ii) Determine the safety of the doses.

Studies could also be conducted into the individual herbal components of the powdered mixture to determine the contribution of each of them to the activity of the powder and also find out if the activity of the powder could be enhanced by the increase or decrease in any of the components.

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APPENDICES

Appendix A

Table A-1 Preparation of culture media

Growth media	Composition	amounts
Nutrient agar	Peptone	5.0g
	Beef extract	1.0g
	Sodium chloride	0.5g
	Agar	12.0g
	Purified water to	1000ml
Nutrient broth	Peptone	10g
	Beef extract	10g
	Sodium chloride	5.0g
	Purified water to	1000ml
Sabouraud agar	Peptone	10.0g
	Agar	20.0g
	Dextrose	40.0g
	Purified water to	1000ml

Appendix B

B-1. PREPARATION OF GLYCEROGELATIN BASE

BP formula for the Preparation of Glycero gelatin base

Gelatin	14g
Glycerin	70g
Water to	100g

14g of gelatin was weighed and mixed with 70g of glycerine, enough water was added was added and well mixed until a clear solution was obtained. The excess water was slowly evaporated off until the weight of the mixture was 100g.

B-2. PREPARATION OF THEOBROMA OIL+10%w/w BEESWAX BASE (100G)

Beeswax	10g
Theobroma oil	90g

10 g of beeswax was weighed and slowly melted, 90g of theobroma oil was added ,melted and well mixed.

PREPARATION OF GELLING AGENTS

In the preparation of the gelling agents, the required quantities were weighed and hydrated in water and stirred until clear gels were formed. The preparations were done at room temperature and stored in a well covered container for further use. In the preparation of 10%w/v pregelatinised potato starch, 20g of potato starch powder was weighed into 200 ml

water and slowly heated at 80℃ over 10 minutes until a gel was formed. The resultant gel was stored in a well covered container for future use.

APPENDIX C

C-1.Calculations for the displacement value of extracts

Displacement value of extracts in glycerogelatin base

Weight of 5 plain glycerogelatin pessaries = 13.0

Weight of 5 medicated pessaries = 11.86

Weight of medicament in pessaries = $\frac{10}{100} \times 11.86 = 1.186$

Weight of base displaced = $13 - \left(\frac{90}{100} \times 11.86\right) = 2.326$

Displacement value = $\frac{\text{weight of medicament}}{\text{weight of base displaced}} = \frac{1.186}{2.326} = 0.51$

Similar calculation was used to calculate for the displacement value of the extract in Theobroma oil + 10%w/w beeswax base.

C.1.1. Calculation for the preparation of 10%w/w G-Rea pessaries with theobroma oil+10%w/w besswax base

Number of pessaries to be produced = 30

Mould size = 2g

Total weight of 30 suppositories = 2g × 30 = 60g

Weight of extract required = $\frac{10}{100} \times 60g = 6g$

Weight of base = 60g – 6g = 54g

C.1.2. Calculation for the preparation of 10%w/w G-Rea pessaries with Glycerogelatin base.

Number of pessaries to be produced = 30

Displacement value of glycerogelatin base =1.2

Mould size =2g

Total weight of 30 suppositories = 2g × 30 × 1.2 = 72g

Weight of extract $= \frac{10}{100} \times 72g = 7.2g$

Weight of base =72g - 7.2g = 64.8g

C.1.3. Calculation for the preparation of capsules

Capsule size used was size '0'

The fill volume of size '0' is 0.68ml

Tapped density of G-Rea extract = 0.62g/ml

Tapped density of lactose powder = 0.87g/ml

Each dose should contain 0.43g of extract but from the tapped density of the extract 0.422g of powder can fill one shell.

Dose was then divided into two shells, each shell having to contain 0.215g of powder.

0.62g of extract occupies a volume of 1ml

Therefore 0.215g = $\frac{0.215}{0.62} \times$ 1ml = 0.347ml

But volume of size '0' shell = 0.68ml

Therefore volume of shell left to be occupied by lactose = 0.68-0.347 = 0.333ml

Tapped density of lactose = 0.87g/ml

1ml of lactose = 0.87g of lactose

$0.333\text{ml} = \frac{0.333}{1} \times 0.87\text{g} = 0.29\text{g}$

Each shell was filled with 0.215 g of extract and 0.29g of lactose

Appendix D : Pictures

Figure D- 4.1. Picture of dried ethanolic extract of powder before milling

Figure D-4.2 .Picture of dried ethanolic extract after milling

Figure D-4.3. Picture of dried aqueous extract of powder

Figure D-4.4. Picture of pessary with glycerogelatin base

Figure D-4.5. Picture of G-Rea pessaries with theobroma+10%w/w beeswax base

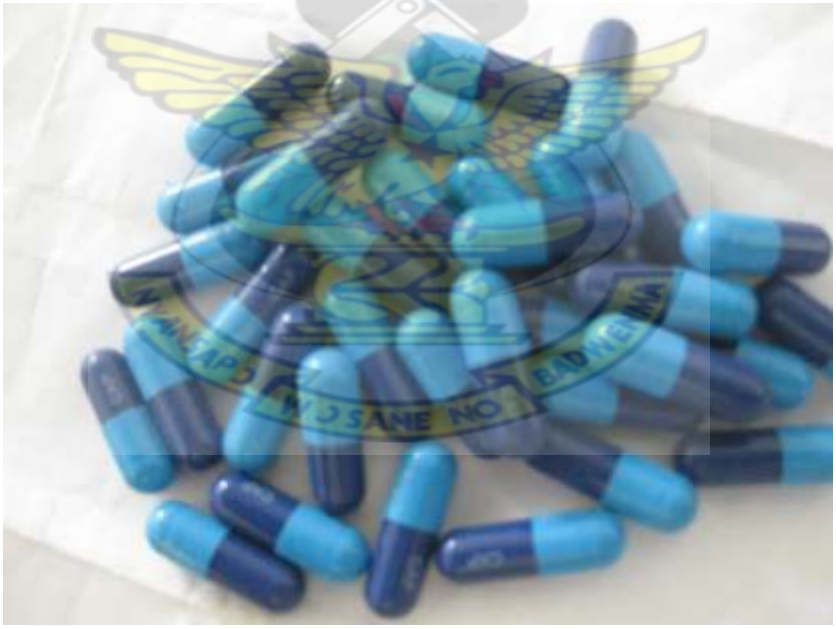

Figure D-4.6. Picture of G-Rea herbal capsules

Figure D-4.7. Picture of 20%w/v HPMC gel with white emulsion colour

Figure D-4.8. Picture of 20%w/v HPMC gel without colour

Figure D-4.9. Picture of 20%w/v HPMC gel with orange colour