FORMULATION OF ANTIFUNGAL SOLUTION USING LOCAL MATERIALS.

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DEDICATION.

This work is dedicated to the creator of Universe and the giver of all good gifts whose name is "**JEHOVAH**" and those who have no helper except Him.

ACKNOWLEDGEMENT.

My thanks go to JEHOVAH who has made it possible for me to reach this point of academic learning despite all the obstacles in my life.

The contributions of my parents, Late Chief T. O Babadiya (of blessed memory) and Mrs. E. O Babadiya are of great values.

The effort of Mr. Babadiya Solomon. A. and Mr. Babadiya Ebenezer G. who brought me out of spiritual and academic darkness can not be forgotten. Therefore, I will ever remain gratëful for all your contributions.

Mr. Gbenga, Mr. Kehinde, and my only sister Bosede did their best for me, I appreciate all of you. May God bless you all accordingly.

Also, the unquantifiable support of my able supervisor Engr. Mohammed Alhansan is something very difficult to offer now a days and I will ever remain grateful to you sir. God will continue to bless you and your family Amen. The effort of all my departmental lecturers can not be underrated in this project.

My encountered with Engr. Akinbode (Late) will be in my memory forever. May God grant him eternal life in paradise.

And to many organizations and individuals who have helped directly or indirectly. May God crown all your efforts Amen.

Finally, Mr. Duro P.O, Abiodun Ogungbenro (Abbey City), Sunday Ogunbiyi, Oyeleye Adekanmi, Olasunkanmi, Adedeji Omotunde, Omonijo, Miss. Sola Ogundoro, and entire members of Jehovah Witnesses in Minna. May God bless you for your efforts and contributions to my success.

ABSTRACT.

Antifungal solutions are antiseptics that inhibit the growth and cause structural disintegration in variety of medical important fungi organisms. Mixture of Elm tree powder, Sugar cane juice, and Sulphur are blended to serve as an antifungal solution however due to its complex nature, the mechanism is not yet understood. This work concentrates on the formulation of different grades of antifungal solution from local materials.

Preliminary analyses/ test conducted on different grades of the sample prepared shows that symptoms disappeared after two weeks of application of the formulated solution on the affected parts with sample D being the most accepted by the users due to its oil in water emulsion of HLB 11.00 that eased its absorption

However the prolonged usage of all the grades resulted into appearance of rashes at the point of application.

Finally, a good blend of antifungal formulated from local materials (Elm tree powder, Sulphur, Sugar cane juice, Palm kernel oil) is effective and will go along way to reduce the cost and provide alternative therapy for the treatment of fungi infection. Hence, the aim of this work has been achieved.

Chapter Six

CHAPTER ONE

1.1 INTRODUCTION.

Virus, Bacteria, Protozoan, Fungi, and other micro organisms have evidently been around since life on earth began. The stunning flexibility of this germ, the simplest of all creatures, has allowed them to survive where nothing else can (John R. Holum).They are found in scalding vent on the ocean floor as well as freezing water of the Artic.

Now these germs are repelling the most concentrated of all assault on their existence antimicrobial drugs. (Kaskel Hummer 1981).

A hundreds of years ago, some microbes or micro-organisms were known to cause illness but no one then living had heard of antimicrobial medicine. A person with a serious infection had little or no treatment to be offered other than moral support by many Doctors. The person's immune system had to fight back the infection on its own. If the immune system was not strong enough, the consequence was often tragic. One thing led to another, people started formulating substance that could cure the infection causes by the micro-organisms.

In the case of Fungi, the most common infection is ringworm, such as athlete's foot, candiasis (candida) (John R.Holum). Serious fungal infections usually afflict only people whose defenses have been weaken by malnutrition, cancer, drugs, or vital infections that suppress the immune system (John R. 1978).

In order to fight this infection, local people have succeeded in formulating the mixture of Elm powder, Sulphur and Sugar cane juice to cure the fungal infections without knowing the right composition, the reason behind it, the mechanism and the principles guiding the mixture and that is what this research work is all about.

1.2 AIMS AND OBJECTIVE.

The primary aim of this research work is to study the mechanism and the principle behind the use and efficacy of fungicide formulated from local materials. And, to as well formulate the best combination of these materials.

The objective is to formulate different blends of these materials and test its efficacy and acceptability to the people and their environs.

1.3 CHEMICAL ENGINEERING ASPECT OF THE PROJECT.

This is purely biochemical, which is an important aspect of Chemotherapy i.e. treatment of disease with chemicals which this could be said to have been one of the contributions of Chemical Engineering to Antimicrobial Chemotherapy.

In the United State alone, some 20,000,000. kg of antibiotics are produced annually, which are exported to other countries (Awake Oct. 22 2003). And since Chemical Engineering is all about the processing of natural resources including raw materials for the benefit of mankind. This work will benefit the medical aspect of economy of any nation. It has a potential of providing employment, foreign exchange and alleviate poverty in our country, through its large scale production.

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORICAL DEVELOPMENT.

Fungi are eukaryotes that poses cell wall, characteristic that classified them as plant. In plant kingdom but lack chlorophyll. There are more than 100,000 known species of fungi. They are heterotrophies that absorb their food through cell wall and plasma membrane. They are non motile and undergo both sexual and asexual and asexual reproduction under favorable condition of moist and dark area. Some live a symbiotic or saprophytic mode of feeding while some live as a parasite. The later are disease causing organisms in plants and animals.

Under favorable conditions, fungi develop spores that are resistance to desiccation (dryness) and other penicillin treatment. Fungi can grow in an environment where PH ranges from 2-9, but optimum PH for most species is about 5-6 while some can grow in concentrated salt or sugar solution (Kaskel Hummer 1981)

2.2 DEVELOPMENT OF ANTIFUNGAL SOLUTION.

Fungal infection has threatened the life of many living creature most especially human. They cause diseases such as athletic foot ring – worm, thrust and lung infection (Kaskel Hummer Daniel).

This calls for an urgent production of Antifungal Chemical. However, the production of Benzylmedazol started in 1944 by Wooly(G.W. Gooday et al 1980). But the first azole marketed for tropical treatment was Clotrimazole in 1959. The interesting aspect of these azoles is that it easily destroyed yeast, dimorphic and filamentous fungi. It has no primary or secondary resistance and completely non- toxic.

In the years following the introduction of Clotrimazole and Miconazole, many chemical analogues of the later were developed; they are Econazole, and Isoconazole, Bitonazole, Oxiconazole etc.

The oral use of Miconazole, is hampered by its gastric intestinal absorption which later lead to the development of Ketoonazole which represented a land mark in the series of azoles antifungal. It is easily absorbed into the blood stream when given through mouth, allowing the therapeutically active level to be build up rapidly and maintained for several hours G.W. Gooday Etal 1980).

Despite the increasing research effort by many Pharmaceutical companies, the most recently synthesized antifungal azoles belong to the triazoles group extracted from juice of higher plants like Elm tree (N.S Egorov 1985)

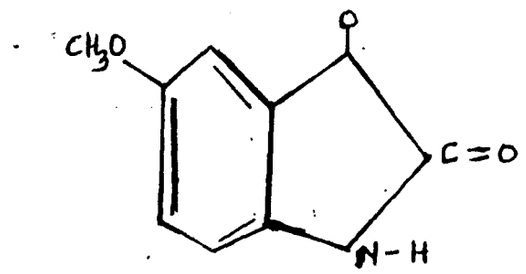
2.3 FORMULATION OF ANTIFUNGAL SOLUTION.

The formulation of antifungal solution can be based on the mutuality relationship that exists between symbiotic Mycorrhizae and root of certain higher plants that stimulated the host plant to secret certain antibiotic such as Phytoncides (N.S Egorov).

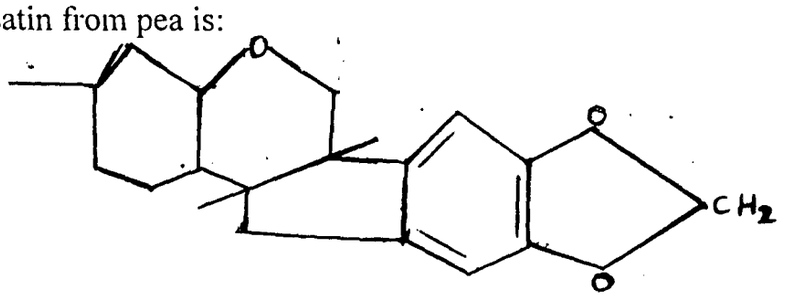
Russian and Soviet scientist studied higher plant juice and came out with findings that certain plant juice had antibiotics characteristics. Their finding with respect to later studied in detail by Tokin in 1951. In 1928, his attention was attracted by a fact that a small branch "**BIRD CHERRY**" plant placed in a glass bell, together with a cup of water containing protozoa, kills the latter in 15 -20 minutes which means that the volatile substrates released by bird - cherry tree have an antiprotozoic effect and named it phytoncides (N.S Egorov 1985)

Elm tree produces phytoalexins as a result of penetration of parasitic fungi into the plant. Point to note here is that Phytoalexins have an antibiotic effect on the parasites producing them.

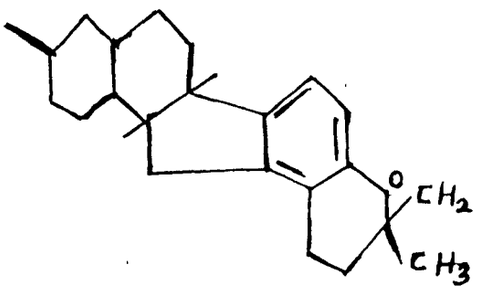
Stability of some higher plants to some fungal diseases is probably connected with formation of the said substances by these plants. Phytoalexins contained 6- Methoxybenzoxazolin, Pisatin and Phaseolin. However, damage tissues of wheat and corn form similar substance that inhibits growth of some bacteria, fungi and also insect feeding upon them (N.S Ngorov). The antibiotic substances isolated from corn are 6- Methoxybenzoxazolin of the following structure (N.S Ngorov 1985)



Pea (Paisium statiumn) and cells of true bean (Phaseolus Vulgaris) infected by a phytopathogenic fungi which are however killed by the antibiotic produced by them (N.S Egorov). The structure of pisatin from pea is:



The structure of Phaseolin from true bean is:



2.4 CAUSATIVE AGENT OF FUNGAL INFECTION.

Fungus provides food for human. Yeast used in making wine, beer and in baking is cultivated strains that are carefully maintained to prevent contamination. Among the basidiomycetes are some 200 kinds of edible mushrooms and about 70 species of poisonous one called the toadstool (Kashel Hummer 1981).

However, these look alike and can only be distinguished by experts. Toxic species such as Amanita Virosa and Phaloides are appropriately called "Destroying Angel" and "Dead Angel' respectively. Ingestion of a single cap of any of these species can kill a healthy person. The trancelike state and colorful visions experienced by those who eat these mushrooms is due to its Psilocùybin contents which is an ingredient chemically related to Lysergic acid diethyl amide (LSD).

Some fungi cause superficial infection in which the infection is centered on the skin, hair or nails other cause systematic infections of tissues, internal organs and may spread through many region of the body.

Ringworm and athlete's foot are example of superficial fungal infection. Candidias is an infection of mucous membrane of the mouth or vagina and is among the most common fungal infections that are caused by a fungus that postulates abundantly in the soil containing bird droppings; a person who inhale the sores may then develop the infection.

More so, people do contact fungal infection through air, drinking water, unhygienic environment and from infected person.

Also, most pathogenic (disease causing) fungi infection only when the body's immunity is lowered. Dry skin is uncomfortable and excessively moist skin is a good host for fungus organism ((N.S Egorov and Awake 2003).

2.5 IDENTIFICATION OF FUNGAL INFECTION.

It is the primary assignment of a medical practitioner to diagnose whether an infection is a fungal infection or not. Once

it is established that it is a fungal infection. The next step is to look for suitable fungicide that can be applied

The mixture of Elm powder, Sugar cane juice and Sulphur has being in used for many years to cure some fungal infections, most especially the one that affects the skin. Establishment of the principles and mechanisms behind the mixture will help to authenticate the efficacy of it.

2.6 TREATMENT OF FUNGAL INFECTION.

The age and sex of the patient together with the knowledge of previous adverse reaction, immune function and renal and liver function must be considered before a final selection of the antifungal is made. In case of tetracycline, it should be avoided by pregnant women and children. Also, co- trimoxazole antibiotic is avoided in pregnancy (kaskel Hummer 1981)

Therefore having considered the above factors and recommendation, the medical practitioner then selects appropriate antifungal with reference to its microbiological and pharmacological properties, adverse reaction and cost.

The production of antifungal from local materials(Elm powder, Sugar cane juice and Sulphur) will reduce the cost and

still meet the required microbiological and pharmacological properties.

2.7 IMPORTANT CONSIDERATION ON THE CHOICE OF ANTIFUNGAL.

Fungal infections are classified as superficial (Skin or Mucous membranes) and systematic for therapeutic purposes.

The former are commonly caused by candida albicans and usually respond readily to topical applications of any anti fungal agent. Systemic fungal infections often occur in a compromised host and can be extremely difficult to cure.

In addition to knowledge about the properties of available antimicrobial agents, important consideration in the choice of effective Chemotherapy are the nature and site of the infection, the known or suspected causative agent (organism), the infected patient and the available antifungal(D.Greewood et al 1985).

2.8 THE NATURE AND SITE OF THE INFECTION

In instance where the nature of the infection can be predicted from the clinical features of the illness, treatment can proceed without isolation of the causative organisms. As in the

prescription of penicillin for acute follicular tonsillitis and lobar pneumonia and the use of ampicillin or co – trimoxazole is prescribed without specific laboratory diagnosis for the treatment of chronic bronchitis caused by pneumococci.

Therefore, if the patient is seriously ill antibiotics therapy must be started on a best guess 'Emprical' basis(G.W. Gooday et al 1980).

2.9 PROPERTIES OF THE RAW MATERIALS FOR THE PRODUCTION OF ANTIFUNGAL SOLUTION.

The raw materials for the production of antifungal are Elm powder, Sugar cane juice, Sulphur and Water.

2.9.1 SULPHUR.

Sulphur is a soft, pale grayish- yellow or greenish – yellow, odorless powder, free from grittiness substance.

It is practically insoluble in water and non alcohol, almost completely soluble in carbon disulphide, the solution depositing the insoluble varieties of sulphur on exposure to light; soluble in hot aqueous solution of alkali hydroxides with the formation of polysulphides and thiosulphites.

Melting point is about 115° C, forming a yellow mobile liquid which become dark and vicious on heating at about 160° C.

Actions and uses precipitated sulphur is a mild antiseptic and parasiticide, preparations of sulphur are applied externally in the treatment of acne (John R. 1978).

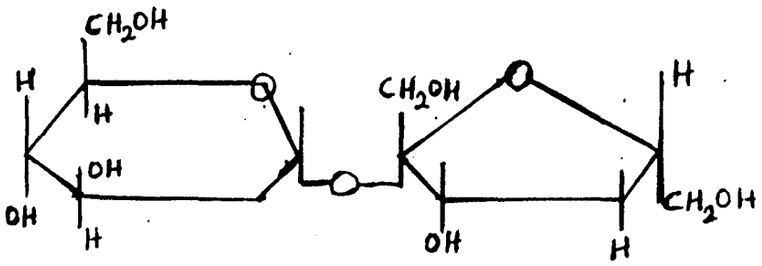
2.9.2 WATER.

Three forms of water are used in pharmacy namely; water for preparation, purified water and water for injection. In this project, we are concerned mostly with water for the preparations potable or freshly boiled and cooled purified water.

Potable water for preparation is drawn from public supply and must be suitable for drinking, water obtained from the supply via local storage tank is unsuitable for this purpose, if such stored water is thinly source, freshly boiled and cooled purified water should be used instead.

Water for preparation is used in preparing medicine such as mixture that are not intended to be sterile but for which water of good bacteriological quality is required (Molly Bloom.1992).

2.9.3 SUCROSE



β –D fructofuranosyl-&-D-glucopyramoside (A sugar obtained from sugar cane juice). Molecular weight; $C_{12}H_{22}O_{11}$

Description: odorless, Lustrous, Dry, Colorless crystal or white crystalline powder with a sweet taste.

Solubility: soluble at 20oc in less than one pant of water and in 370 pants of alcohol.

Hydroscopic: it absorbs insignificant amount of moisture at relative humidity up to about 85% but under damper condition it absorbs substantial amount.

Action and uses: sucrose is used as a sweetening agent and as a demulcent solution of sucrose in concentrations of less than 65% m/w Ferment, but more concentrated solutions, such as syrups have an osmotic pressure sufficiently great to inhibit the growth of most bacteria and fungi (John R Holum 1978).

2.9.4 ELM POWDER:

Prestocarpus Soyauxii.

This forest tree may be distinguish from others by its rather narrower leaflets with numerous very thin, close-set lateral nerves running at a wide angle to the mid rib. Different localities have different names for this tree;

Tribe	Name
Yoruba	Osun pupa
Edo	Akume
Igbo	Awo
Efik	Ukpa
Boki	Boku
Nupe	Iza

Elm tree extends from south-western Nigeria to Zaire and habitat is always rain forest.

Description: it is about 30m high and 2.5m girth. Bole straight, cylindrical, sometimes with sharp buttresses up to 4m high. Back; dark brown, rough, slash whitish, immediately exuding red juice when scratched.

Leave with a slightly angled common stalk 10-20 cm long, 10:17 leaflets 6-10cm long by 2-3cm broad, elongated elliptic, acuminate, sometime with the mid rib projecting or notched at the top, round at the base , slightly hairy beneath, often with 15 or more pairs of very thin rather vague lateral nerves, terminal leaflet broader, lower leaflets smaller and the bottom ones ovate, stalks of leaflets under 6mm long.

Flowers (June, September- October) yellow; in lax panicles about 15cm long the central talk and branches of the panicle densely covered with very short rusty hairs; individual flower with slender stalks hairy, longer than the calyx and with two slender brateoles at the apex about 7mm long including the teeth, standard- petal about 12mm long.

Fruit (Aug.—Jan.) flat and papery, across at first densely velvet with very short hairs, finely hairy when ripe

Uses: some local people, most especially in the northern and western parts of Nigeria always use the powder obtained from the stem of elm tree after carrying out the pounding of the stem by pestle and mortar to cure fungi infection.

In addition, they use it to rub a new born baby to kill germs that the baby may have.

2.9.5 PREPARATION OF ANTIFUNGAL SOLUTION.

The solution of Elm powder, Sugar cane juice and Sulphur will be in cream form since it is intended for external use only. These materials are blended together thoroughly and injected into cream solution.

Creams are vicious semi-solids and are usually either oil in water or water in oil (oily cream). Creams are used to apply solutions or for dispersion of medicaments to the skin for therapeutic or prophylactic purpose where a highly occlusive effect is not necessary.

In creams applied topically for therapeutic effect, it is desirable that the active substance should be released at the skin surface and should penetrate at a suitable rate in sufficient amount to maintain an effective concentration at the site of the action.

Also, the outermost horny layer of the epidermis provides the principal barrier to medicaments and vehicles. Some

antifungal may also be inculcated inside soap tablets (John R Holum).

2.9.6 FACTORS WHICH AFFECT THE PENETRATION OF THE SUBSTANCE THROUGH THE SKIN INTO THE BLOOD STEAMS.

(1) Condition of the Skin:- Skin present a barrier to absorption which can be considerably reduced when the skin is damaged or is in the diseased state. The rate of absorption is also influenced by the age and environmental effect on the skin temperatures and surface humidity. In planter and palmer areas of the body surface Keratin layers are thick absorption rates are slow.

(2) Physico-chemical characteristic of the active substance:-

Both penetration and thermodynamic activity (chemical potential) of an ionic substance are influenced by its ionization consent and by the PH of the skin and aqueous vehicles.

An increase in the concentration for an active substance in a vehicle usually produces an in increase in the amount absorbed percutaneously.

(3) Effect due to vehicle :-

The diffusion constant of drugs in a vehicle is inversely proportional to viscosity. The state of hydration of the skin can also be affected by the vehicle. Increase hydration is achieved clinically by reducing evaporation by means of an occlusive layer.

2.9.7 EMULSIFYING AGENT.

Emulsifying agent prevent coalescent of the dispersed globules in emulsified system by forming barriers at the interfaces.

They may also facilitate the initial dispersion of globules by reducing interfacial tension. The ideal emulsifying agent of pharmaceuticals purposes to be stable, inert, and free from toxic and irritant properties it should be odorless and colorless a and low concentration should also produce a suitable emulsion of desired type.

Emulsifying agent can be classified into three groups; Synthetics substances, natural products and finely divided solids. Many traditional emulsifying agent are derived from natural product such as plants and animals with complex and undefined or variable composition. microbial contamination and hydrolysis on storage of so

many natural emulsifier is their major limitation(Pharmaceutical codex).

2.9.8 THE HLB SYSTEM.

The balance between the Hydrophilic and Lipophilic moieties of surface active molecules has been used as the basis for a more rational means of selecting and classifying emulsifying agent than the empirical method of traditional used. The relationship between HLB numbers and surfactants properties is as follows:

HLB RANGE	PROPERTY
4 – 6	Emulsifying agent (w/o)
7 – 9	Wetting agent
8 -18	Emulsifying agent. (o/w)
13 -15	Detergent.
10 – 18	Soluble agent.

(Courtesy: Davidson's Principle & Practical medicine)

Many oil and waxy materials used in emulsion have been given a required HLB value, determine by experiment to facilitate the selection of the appropriate emulsifier.

Example of require HLB value are given below:

Required HLB values for oils and waxes emulsion type;

	o/w	w/o
Bee wax	12	5
Castor oil	14	-----
Cety alcohol	15	------
Cotton seed oil.	9	------
Paraffin hard.	10	4
Paraffin liquid.	12	4
Paraffin soft.	12	4
Stearic acid.	16	-----

(Courtesy: Davidson's Principle & Practical medicine)

It is often to obtain blend of emulsifier of a greater efficiency from a mixture of two or more emulsifier.

This is better than a single emulsifier. It is often necessary to prepare series of emulsifier in order to determine the optimum combination.

Moreover, the HLB calculation does not take into account the concentration of emulsifier to be used.

2.9.9 EMULSIONS.

An emulsion consists of two immiscible liquid phases one of which subdivided and uniformly dispersed in the other. The system is stabilized by the presence of an emulsifying agent. The dispersed liquid, or internal phase, usually comprises globules of sizes down to 0.1micrometer which are distributed within the external or continuous phase.

2.9.9.1 PREPARATION OF EMULSION.

Before an emulsion is prepared the oil soluble and water soluble constituent are separated, dissolve if necessary in the appropriate phase and a suitable emulsifier agent is selected for the type of emulsion required(water in oil or oil in water) this is also dissolved either in the aqueous or the oil phase. Ideally, the internal phase volume, lower phase volume increase the tendency to "cream or sediment".

Emulsions for external application [creams, application, ointment and lotions] often include waxy solids.

2.9.9.2 EQUIPMENT

The mortar and pestle are simple and inexpensive equipment for the preparation of small quantity of emulsions.

Containers for emulsions:- Emulsions for external use should be label "For external use only" and should be supplied in wide mouthed bottles fitted with plastic screw closures which provide airtight seal, metal caps should be avoided. Containers should be well fitted to minimize evaporation.

2.9.9.9.3 PRESERVATIVES.

Micro- organisms can rapidly proliferate in emulsified systems with high water content, particularly if carbohydrate, proteins or steroidal materials are also present.

And ideal preservative should possess effective antimicrobial activity against a broad spectrum of micro-organisms over- a wide range of conditions of temperature and PH free from toxicity and sensitizing effects at the effective concentration.

Pharmaceutical preparation intended for parenteral and ophthalmic administration, for introduction into body cavities as irrigations, or application to wounds must be specially prepared and

sterilized. If presented in multi-dose containers an effective antimicrobial agent must be included in the formulation of other forms of medicines in which micro organism are capable of multiplying.

In particular those intended for Oral and Topical administration, preservatives commonly used in chemical and pharmaceutical product include; alcohol, benzoic acid, sulphurdioxide acetate etc.

CHAPTER THREE.

3.0 EXPERIMENT:

All experiments were carried out at laboratory scale.

3.1 MATERIALS AND METHODS.

The materials used for the experiment include laboratory equipment and reagents.

3.2 EQUIPMENT.

(1) 2 – Liter laboratory sized volumetric flask.

(2) Drying oven, Stainless dish, Petri dish, Scapula

(3) Mortar and Pestle, Sieve.

(4) Top electronic weighing balance.

(5) Volumetric analysis equipment, steam bath.

(6) Sample bottles.

3.3 REAGENT USED

(1) Distilled water.

(2) Detergent.

(3) · Sugar cane juice, Palm Kernel oil, Sulphur, Glucose, and Elm powder.

3.4 METHODOLOGY.

Standard techniques for the medicament preparation Are used as given by organization of Pharmacologist and the procedure were followed accordingly.

3.4.1 DRYING OF ELM POWDER.

The elm powder was dried in the sun for several hours. This was done to obtain an effective crushing and sieving of the materials.

3.4.2 CRUSHING OF SULPHUR AND DRIED ELM POWDER.

Crushing is the operation of reducing to smaller particles of bulk or larger materials. Both sulphur and elm powders were crushed separately by the use of mortar and pestle to reduce to smaller particles.

3.4.3 SCREENING OF THE PARTICLES

Screening is the separation of mixture of various sizes of the particles into different portions by means of screening surface. Here both the grind sulphur and elm powder were sieved to obtain particle sizes of 500um and 250um respectively.

3.4.4 CRUSHING OF SUGAR CANE.

PROCEDURE:-

The following are steps to obtain sugar cane juice.

(1) The back of sugar cane straw were peeled off and crushed into pieces.

(2) It was then pass through a screw press to release the juice.

(3) The juice was then analyzed for its concentration.

(4) Regulation of the concentration of the juice was necessary, if the concentration is less than 65%. This was done by dehydration.

(5) The juice was then filtered with white clean and sterilized cloth to remove unwanted particles.

3.4.5 DETERMINMATION OF CONCENTRATION OF SUCROSE IN SUGAR CANE JUICE.

PROCEDURE:-

(1) A stainless dish was dried in an oven set at 80oc for 1 hour.

(2) It was then cooled in a desiccators and weighed(W1)g

(3) The sample was then put into a dish and weighed (W2)g

(4) The dish with the content was dried in the oven at temperature between 80°C and 150°C for 2 to 4 hours until constant weight was obtained.

(5) The dish was then transferred to desiccators allowed to cool and quickly re-weighed again and that was recorded as (W3)g. the lost in weight of the sample during drying is the moisture content calculated as follow:

Moisture content = (W2-W3) g

Amount of solid sucrose = (W3 –W1)g

$$\text{Concentration of Sucrose g/dm3} = \frac{W3 - W1}{W2 - W3}$$

3.4.6 PREPARATION OF THE ANTIFUNGAL SOLUTION.

Elm tree powder, sugar cane juice, Distilled water, Sulphur, and emulsifying of desired quantity were mixed as follows:

(1) Solution of elm tree powder, sulphur powder was made by addition of appreciable amount of Sugar cane juice having a concentration of above 65%.

(2) The desired emulsifying agent of HLB of 9.5, 10.0, 10.5, and 11.0.were then prepared and kept in liquid form (50oc).

(3) A basis of 30g of antifungal solution was used for 4 different samples.

(4) The solution was prepared in four different sample bottles having emulsifier of HLB. Ranging from 9.5 to11 respectively.

(5) Each bottle contains 5g of elm powder, 20g of sulphur and 5g of emulsifier.

(6) . The solution was thoroughly mixed to obtain homogenous mixture.

(7) The sample bottles were then labeled according to the standard way of labeling drugs for external use and fitted with screw closure which provide air tight seal. The combinations of different grade of anti fungal solution produced are presented in table 4.1.

3.4.7 CLINICAL / MARKET SURVEY.

Sample.opinion was taken for each grade of the blend and result is as presented in table 4.3.

CHAPTER FOUR

4.0 RESULTS.

TABLE 4.1: COMPOSITION OF VARIOUS BLENDS OF ANTIFUNGAL SOLUTION. BASIS: 30 CM^3 OF EMULSIFYING AGENT.

	HLB	%palm kernel oil	%paraffin wax	Palm kernel vol.(cm3)	Paraffin wax vol.(cm3)	Wt.of sulphur(gm)	Wt. of elm powder (gm)
A	9.5	83.3	16.7	25.00	5.0	20.00	5.00
B	10.0	66.7	33.3	20.00	10.00	20.00	5.00
C	10.5	50.0	50.00	15.00	15.00	20.00	5.00
D	11.0	33.30	66.70	10.00	20.00	20.00	5.00

Constants:

Particle size of elm powder and sulphur 500um.
Sucrose concentration = 87.78%
Sulphur powder = 20.00g
Elm powder = 5.00g

TABLE :4.2 PARTICLE SIZE 250um AND SUCROSE BASIS: 30 CM^3 OF EMULSIFYING AGENT.

	HLB	% palm kernel oil	% paraffin wax oil	Palm kernel oil (cm^3)	Paraffin wax (cm^3)	Elm powder (g)	Sulphur(g)
E	10.5	50.0	50.0	15.0	15.0	15.0	20.0
F	10.5	50.0	50.0	15.0	15.0	10.0	15.0
G	10.5	50.0	50.0	15.0	15.0	5.0	10.0

CHAPTER FIVE.

5.0 DISCUSSION OF RESULT.

A detail investigation on the formulation of antifungal solution had been performed. Different grades were formulated, produced and tested. The results obtained are presented in table 4.1 to table 4.3.

In table 4.1 it can be deduced that from grade A to D formulated, each contains 5g and 20g of elm powder and sulphur respectively and 5g of emulsifying agent.

The emulsifier in sample A is a combination of 25cm^3 of palm kernel oil and 50cm^3 of paraffin wax given an HLB of 9.5. While sample B, C, and D have a combination of 20, 15, and 10cm^3 of palm kernel oil and 10, 15 and 20cm^3 of paraffin wax and HLB of 10, 10.5, and 11 respectively.

However, particles size of elm powder and sulphur and concentration of sucrose were kept at 500um and 87.78% respectively. This is in agreement with standard preparation of antifungal solution presented in the literature(Pharmaceutical Codex).

Table 4.2 shows that emulsion used in the preparation of samples E, F, and G have the same HLB value of 10.5 and 20g , 15g,

10g, and 5g of sulphur and elm powder respectively while particle size were kept at 250um and no sucrose solution was used.

Preliminary test conducted on different grade of solution shows that symptom disappeared after 2 weeks of application of antifungal solution on the infected part, with the sample grade D being the most accepted by the people due to its oil in water (o/w) emulsion of HLB of 11 that its ease the absorption into the body. There is also a clear indication that sample E, F and were rejected by the people due to their low sulphur content and high paraffin wax.

In all the grades, prolonged usage resulted into appearance of rashes at the point of application (table 4.3)

CHAPTER SIX.

6.0 CONCLUSION:

Different grades of antifungal solution were formulated and tested. The effectiveness of the solution depends on the HLB value of emulsion and a good blend of the local materials. Analysis on the formulated blend shows that grade D is the most accepted by the users and that the prolonged usage of all the blends resulted in appearance of rashes on the point of application.

The best grade of antifungal solution produced using local material is safe to handle and environmental friendly and will also go long way to reduce the cost and provide alternative therapy for the treatment of fungi infections.

6.1 RECOMMENDATION.

Since the prolonged usage of antifungal solution resulted into appearance of rashes at the point of application it is recommended that further research work should be carried out to eliminate this side effect completely.

APPENDIX:

SAMPLE CALCULATION.

The calculation of the required amount.

Here , X is the percentage of palm kernel oil and 100 - X is the percentage of paraffin wax.

Palm kernel HLB = 9

Paraffin wax HLB = 12

Therefore for HLB of emulsifier, we have:

$$HLB = \frac{X}{100} \times 9 + \frac{(100- X) \times 12}{100}$$

$$= 0.09X + (1- 0.01X) \times 12$$

$$=0.09X + 12 - 0.12X \text{----------(1)}$$

Using equation 1 for HLB of 9.5

$$9.5 = 0.09X + 12 - 0.12X$$

$$9.5 - 12 = 0.09X - 0.12X$$

$$-2.5 = -0.03X$$

$$X = -2.5/ -0.03$$

$$X = 83.33\% \text{ of palm kernel oil.}$$

Therefore, 100 – 83.33 = 16.67% of paraffin wax.

FOR HLB = 10

$$10 = 0.09X + 12 - 0.12X$$

$$10 - 12 = 0.09X - 0.12X$$

$$-2 = -0.03X$$

$$X = -2/ -0.03$$

$$X = 66.70\% \text{ of palm kernel oil.}$$

Therefore, 100 – 66.70 = 33.30% of paraffin wax.

FOR HLB 10.5

$$10.5 = 0.09X + 12 - 0.12X$$

$$10.5 - 12 = 0.09X - 0.12X$$

$$-1.5 = -0.03X$$

$$X = -1.5/-0.03$$

$$X = 50.0\% \text{ of palm kernel oil.}$$

Therefore, 100 – 50% = 50 of paraffin wax.

FOR HLB 11

$$11 = 0.09X + 12 - 0.12X$$

$$11 - 12 = 0.09X - 0.12X$$

$$-1 = -0.03X$$

$$X = -1 / -0.03$$

X = 33.30% of palm kernel oil.

Therefore, 100 – 33.30 = 66.70% of paraffin wax.

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