TABLETING PROPERTIES OF THE BARK AND LEAF POWDERS OF *AZADIRACHTA /NDICA* (A. JUSS)

BY

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my research work carried out under the supervision of Prof. A.B. Bangudu and Dr. Y.K.E. Ibrahim

The work has not been presented in any previous application for a higher degree. All sources of information are specifically acknowledged by means of references.

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This work is dedicated to my family and that of Isah Babangida.

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Finally, I am grateful to my wife and the Isah Babangida family for the understanding and care throughout the period of the study.

ABSTRACT

Azadirachta indica (neem tree) is a well known medicinal plant found particularly useful in the treatment of malaria. It is readily available in the northern part of this country. Despite the wide usage of this plant in malarial chemotherapy, the dosage has not been standardized. It has therefore become imperative to commence work towards formulating thi3 plant or its parts into a suitable dosage form. In this study, attempts were made into formulating the barks and leaves into tablets.

To investigate the tableting properties, the physico-chemical characteristics of the bark and leaf powders of the plant such as mean particle size and size distribution, particle morphology, flow properties Gangle of repose, flow rate and Carr's index as well as moisture contents, Using standard procedures. Compact characteristics such as thickness, hardness, friability, and dis-integration times of the tablets compressed from these powders by wet granulation and direct compression methods were investigated. The effect of various binders and granule size fractions on the characteristics of tablets produced by wet granulation method and that of diluents on tablets produced by direct compression were determined.

Microbiological analyses such as microbial contamination levels and flora determination, and evaluation of the antimicrobial property of the plant was also carried out. Tests were also done to detect the presence of some indicator organisms.

In order to determine an effective dosage regimen for a rational therapeutic use of this plant in malaria treatment, pharmacological screening was carried out. Using a rodent

parasite, *Flasmodium yoelii nigeriensis,* in swiss albino mice, the plant was screened for its curative, prophylactic and suppressive application in malaria.

Investigations of the physico-chemical properties of the powders of the bark and leaf indicated that these powders were mostly fibrous in nature, with relatively poor flow characteristics. Tablets produced by direct compression from the leaf powders were generally unacceptable when subjected to quality control tests. Those lablets produced from the bark and binary mixtures of the bark and leaf powders were of better quality. Avicel pH 101 at low concentrations produced better quality tablets compared with the other diluents: lactose and Starch Rx 1500.

Tablets produced by wet granulation method were generally better than those of the direct compression method. Maize starch and polyvinyl pyrrolidone produced good quality tablets when used as binders. On the other hand, tablets produced from the bark powders using methylcellulose as binder were generally friable with short disintegration times. The granule size fraction used was also found to have considerable influence on the resulting compact characteristics. For example, particle size fraction less than 500 µm but greater than 150 µm gave the best quality tablets.

Microbiological investigations of the bark and leaf powders indicate moderate contamination with bacterial and fungal organisms. The predominant bacterial contaminants were spore-forming and non-sparing Bacillus while Aspergillus and mould were the dominant fungal organisms. No indicator organism *{E. coli.. Ps. aeruginosa.. Salmonella* spp, *Staph, aureus* and *C. albicans)* was detected. Aqueous and ethanolic extracts of *Azadirachta indica* exhibited significant inhibition on the gram positive test organisms *{B. subtilise Staph, aureus), C. albicans* and *E. coli.*

Only little activity was exerted against P*s. aeruginosa* by the ethanolic extract.

Pharmacological screening of the bark and leaf powders of *Azadirachta indica* showed that the plant has useful prophylactic and suppressive antimalarial activities but has very little curative property. The leaf powder exhibited higher degree of antimalarial effect than the bark powder. Extrapolation of the pharmacological data from the albino mice indicated that an adult human being may need to ingest about 155 tablets of a 300 mg weigth per day for significant therapeutic improvement in malaria infection.

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CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

Medicinal plants still remain a major source of drugs in the treatment of various ailments especially in the third world countries (Phillipson *et al*, 1989). While many of the plants contain principles whose effects can be demonstrated pharmacologically and the action of the whole plant or its extract is traceable to some of its isolated constituents, it has not been possible for most plants, to relate their pharmacological activity to any of their constituents. The situation is more complicated by the frequent use of drugs in combination, of which the supposed active constituents have not been elucidated. In some other instances, the active constituents are so complex that complete and partial synthesis has not been possible, hence the whole plant or its extract continue to be used therapeutically. This is the case with digoxin and senna leaves which have been compressed into tablets.

Malaria is the most prevalent fatal infection in Tropical Africa, killing more than one million people every year (WHO, 1987). Remedy sought from local plants have been in the increase to:

1. reduce both mortality and morbidity;
2. to serve as a substitute to the synthetic antimalarials currently resistant to *Plasmodium falciparum*; and
3. for its relative cheapness and availability.

The most common and abundantly used of the plants in the treatment of malaria is Neem tree (*Azadirachta indica*). The antimalarial property of this plant has scientifically been proved (Iwu *et al*, 1986). Because of its widespread use, it is desirable to formulate this plant into an appropriate and standardized dosage form for use by the populace.

1.1 MEDICINAL PLANTS: A BRIEF HISTORICAL BACKGROUND

Since the dawn of history, man has been puzzled by the sudden ravages made by disease, and has attempted in many ways to find cures for his ailments. Such cures have passed through various vicissitudes, from the earliest stages of fanciful and even repulsive remedies obtained from vegetable and animal sources, and gradually to the modern era of rational chemotherapy. The foundation for therapeutics has been severally laid in various countries, at various periods of time.

Perhaps, Chinese medicine was about the earliest, as available records dated about 2500 BC give us an idea of the medical knowledge of the Chinese in such primitive times. Ephedrine was used in China for over 5000 years before being introduced into western medicine in 1924 (Chen *et al*, 1930).

Senna obtained from the dried leaflets of *Cassia acutifolia* was introduced into Arabian medicine as early as the ninth century. Crystalline senna glycosides, Sennosides A and B, and concentrates of senna pods standardized by chemical or biological assay are also available which are more stable and reliable than the preparations of senna leaf (McClure *et al*, 1957). Quinine which is the chief alkaloid

of the bark of cinchona tree is indigenous to certain regions of South America. It was made into powder and given as a beverage, to cure fever.

Digitalis or foxglove, was mentioned in 1250 BC in the writings of Welsh physicians. Digoxin and digitoxin, the two principal glycosides of *Digitalis purpurea* and *Digitalis lanata* have found uses in cardiac problems since some years ago. *Rauwolfia serpentina* (Benth) which is indigenous to India and other neighbouring countries was found useful in the treatment of psychotic patients (Klein, 1954). Cod-liver oil obtained from *Godus morhua* is a source of vitamins A and D (David *et al*, 1954).

The Babylonians, who specialised in astronomy and astrology laid great stress on the influence of the stars. The use of iron in anaemia was in the first instance, due to the belief that Mars influenced the blood and iron. Its real therapeutic significance was not known until centuries later. Egyptian medicine which is also very ancient, probably borrowed from Babylonian and Assyrian sources. The **Ebers Papyrus** which is dated about 1500 BC gives a collection of drugs prevalent in Egypt at that time, with their classification and their uses. Some of the drugs employed now, such as castor oil and pomegranate bark, are mentioned in this papyrus (David *et al*, 1954).

Greek medicine is said to be the father of modern medicine and therapeutics. Hippocrates, in the 5th Century BC, separated medicine from religion. He gave rational medicine the form it holds today by laying down certain principles on which modern medicine is built.

1.2 MALARIA

Malaria is primarily a tropical and subtropical protozoan disease characterised by rigour, pyrexia and fever following the bite of Anopheles mosquito. The causative protozoans belong to four species of the genus Plasmodium namely, *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*.

1.2.1 LIFE CYCLE OF THE MALARIA PARASITE

Though morphologically different, the development of the four species of human plasmodia follow the same pattern. The infective form of the parasite is the uninucleated spindle-shaped cell called 'sporoziote', found in the salivary glands of the infected mosquito. The sporozoite are injected into the blood stream of man through the proboscis when the mosquito squirts some anticoagulant saliva before beginning to suck.

The sporozites can survive in the blood for not more than an hour and within thirty minutes of inoculation, they gain access to the liver where they undergo growth and multiplication in the parenchymatous cell. This is the pre-erythrocytic tissue schizogony and is completed towards the end of the incubation period of the infection when large numbers of the tissue merozoites from ruptured tissue schizonts are released into the blood.

The tissue merozoites then invade the red blood cells. On entering the erythrocyte, the growinmg parasite (now called trophozoites) take on ring shape. As it matures, the central vacuole dissappears till it completely fills the whole red blood cells. The trophozoites ingests the protein portion of the haemoglobin. Thereafter nuclear fission

results in the formation of schizonts and subsequently into merozoites. The erythrocytes then bursts releasing merozoites, pyrogens and haem from haemglobin into the blood stream manifesting as bursts of fever.

Some erythtocytic forms develop into two types of sexual parasites called gametocytes. The male is smaller hence called microgametocytes. The gametocytes are not capable of further development in the human host but on being picked up by blood sucking mosquitoes, the next stage commences. In the stomach of the mosquito, the gametocytes are freed from the erythrocytes. The male gametocyes divide into three, producing eight threadlike microgametes. Combination of one microgametocyte with a female gametocyte results in the formatin of a zygote. This motile zygote (Ookinete) burrows into the stomach wall and assumes a spherical shape called OOcyst. It is within this oocyst that thousands of sporozoites are produced through multiple fission. On their release from the OOcyst (which raptures after a development period of about 12 days in the mosquito), the sporozoites find their way to the salivary glands.

1.2.2 CHEMOTHERAPY

Malaria chemotherapy is based on the principle of selective toxicity. Antimalarials are agents used to inhibit the development of plasmodia and are administered with the aim of totally eradicating the parasite from the host. They have selectively higher affinity for and lethal effects on the parasites as compared with the host cells. The choice of the antimalarial agent is governed by the aim of the therapy

(whether for prophylaxis or for treatment), the species of the parasite and in these days of widespread drug resistance, also on the geographical location of the patient.

Casual Prophylaxis

This is the prevention of infection after the bite of an infected mosquito. Here, the drug must be able to destroy the sporozoites before they gain entry into the liver cells. Such a drug does not presently exist. Casual prophylactic agents are also used to kill exoerythrocytic stages of the parasites in the liver. Primaquine is a casual prophylactic but is rarely used because of its serious side effects. Pyrimethamine and proguanil which are safer are mre commonly employed as prophylactic agents in malaria chemotherapy.

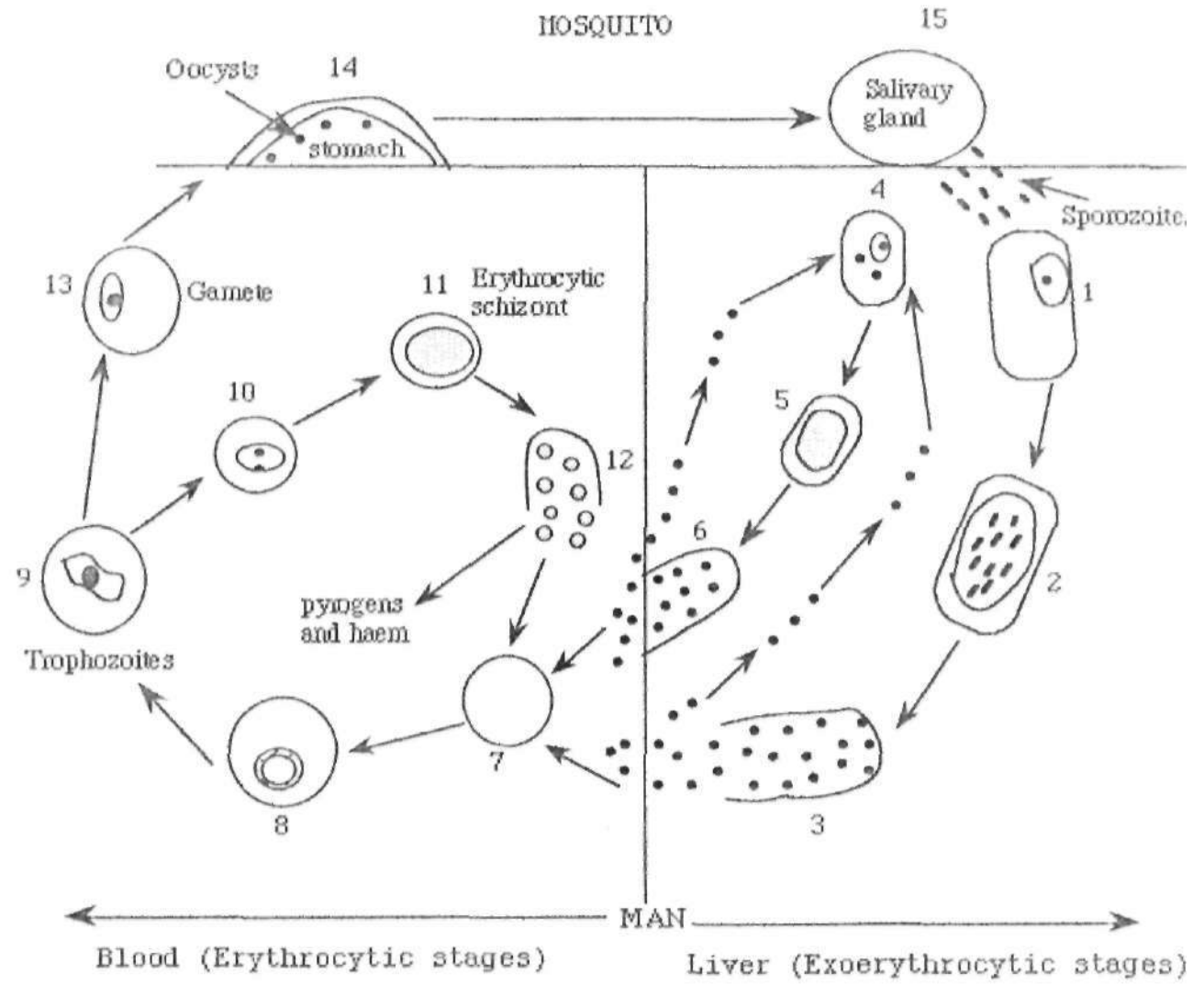
Clinical Cure

The aim of clinical cure is to terminate a clinical attack. This is effected by using blood schizonticides of which the 4-aminoquinolines are the drugs of choice. The dihydrofolate inhibitors (proguanil and pyrimethamine) which are also schizonticidal have very slow onset of action.

Radical Cure

As the name implies, radical cure involves the total elimination of both the erythrocytic and exoerythrocytic forms of the parasite without allowing any relapse. Radical cure in *P. falciparum* is achieved by the use of the 4-aminoquinolines. In *P. vivax* however, primaquine is the drug

of choice though it can be used concurrently with chloroquine.



1-3 : Pre-erythrocytic stages
4-6 : Paraerythrocytic stages (not found in *P. falciparum*)
7-12 : Asexual erythrocytic stages
13 : Gamete (in the blood)
14 : Oocysts in mosquito stomach
15 : Sporozoites in mosquito salivary gland

Fig. 1 : The Life Cycle of Malaria Parasite in Man (1-13) and Mosquito (14-15)

1.3 *AZADIRACHTA INDICA* (Fam: Meliaceae)

1.3.1 SOURCE

Azadirachta indica A. Juss (*Meliaceae*) is a native of Asia which has now naturalised in West Africa. The neem tree, known locally as "dogon yaro" or "darbejiya" in Hausa, "aforo-oyimbo" in Yoruba and "ogwu-akom" in Ibo, is widely cultivated throughout Nigeria as an ornamental plant. It is a drought resistant plant and therefore grows well even in the arid parts of the country, growing up to 25 m high in some places but occurs mostly as a medium sized tree.

1.3.2 PHYTOCHEMICAL CHARACTERISTICS

Azadirachta indica is an evergreen tree up to 25 m tall, grey bark and scaly trunk, up to 60 cm in diameter, crown with upright or spreading branches and dark-green, pinnate leaves up to 30 cm long. Leaflets are 7-17 in number and are directly or almost opposite, somewhat curved, oblong-lanceolate and up to 7 cm long with a noticeable onion smell. Leaf margins are strongly serrate with cineate base and unequal. Flowers are white and fragrant, in large ponicles. Fruits are berry-like, oval-oblong, yellow to reddish-brown and only slightly fleshy and are single seeded (Randhawa, 1965).

1.3.3 MEDICINAL USES

One of the most important uses of neem plant is its application in malaria therapy. A decoction of the leaves or of leaves and stem bark is used in the treatment of fevers by hydrotherapy or inhalation (Sofowora, 1982). Alcoholic extracts or aqueous infusions of the leaves or stem bark is

claimed to be effective in malaria attacks when there is allopathic antimalarial drug-resistant plasmodium strain (Iwu *et al*, 1986). The antimalarial effect of this plant has been estimated to be equivalent to half the therapeutic doses of chloroquine sulphate on dry weight basis (Ade-Serrano, 1982).

In India, leaves are applied in the form of poultice for boils and the decoction is recommended in the treatment of ulcers and eczema (Indian Pharm. Codex, 1953).

In Nigeria, the alcoholic and aqueous extracts are widely used not only to cure malaria, but also to cure jaundice, skin diseases and also as a purgative. The oil from the seed is used as an ingredient in remedies for hemorrhoids (Iwu *et al*, 1986).

The fruit is used as a laxative and for the treatment of urinary tract diseases, intestinal worm infestations and leprosy. The stem and roots are efficiently used as chewing sticks and mouth inflammation can be reduced by including the extract in dental preparations. Powdered plant parts and extracts have been incorporated into toothpaste and tooth powders (Lewis and Elvin-Lewis, 1977).

1.3.4 OTHER USES

Some active ingredients of the seeds and leaves of the tropical neem tree, especially the tetranortriterpenoid: azadirachtin, influence the feeding behaviour, metamorphosis, fecundity and fitness of numerous insect species belonging to various orders. In recent years, a number of neem products have been applied in laboratory to test their suitability for practical pest control purposes.

When aqueous extracts of neem leaves at a concentration of 0.5% was sprayed fortnightly on onions in the field in Mauritius, damage by *Liriomyza trifolii* was considerably diminished. Yields in the neems treated were four to six times higher than in the control plots (Fagoone *et al*, 1984).

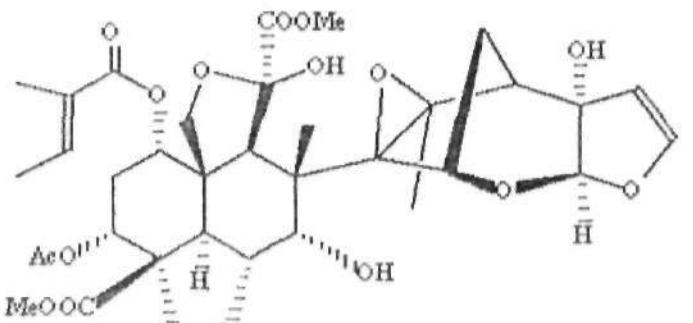
In rural India, it has been an age-old practice to mix dried neem leaves with stored grains and to place leaves in the folds of woollen clothes to ward-off insect attack. This practice has been inherited as a tradition. Pruthi *et al* (1944) reported that neem leaves were spread in 5 to 7 in thick layers in grains and neem fruits were crushed on the inner surfaces of the grain cribs. Mixing of neem leaves with wheat, rice and other grains at a concentration of 2-5% is even now a common practice in many villages in India and Pakistan.

The neem seed and other parts of the tree contain insecticidal tetranortriterpenoids such as azadirachtin (Butterworth *et al*, 1968), meliantin (Levie *et al*, 1978), and salannin (Warthen *et al*, 1978) which act in insects as antifeedants, as insect growth and development regulators, or even as toxicant. The most potent compound of all is azadirachtin.

1.3.5 THE CHEMISTRY OF NEEM TREE

As is usual with such botanical material, the bark, heartwood, leaves, fruits, and seeds have been examined to some extent by chemists, but it is the renewable parts: the seeds and leaves that have received the most attention. In spite of this, our knowledge of this plant is only frag

mentary. The impetus towards the investigation of triterpenoids of neem was provided by the first isolation of a substance with insect feeding-deterrant properties (Butterworth *et al*, 1968). This substance, azadirachtin (structure 1), is a highly oxidised triterpenoid. Today, there is an increasing body of knowledge of these fascinating and structurally complex substances. However, the study of the other types of compounds from the neem tree has hardly begun.



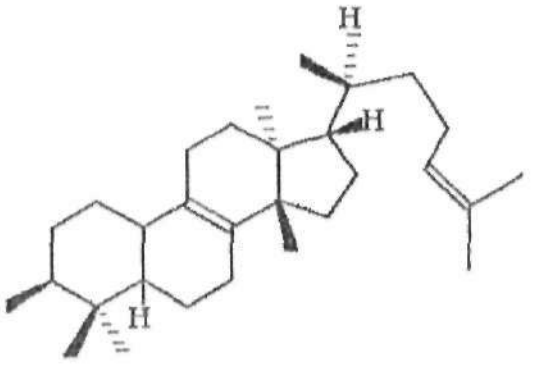
azadirachtin (1)

The seed oil which is usually extracted by steam or solvents from the crushed seeds consists chiefly of triglycerides and large amounts of triterpenoids. Gas chromatographic analysis showed that the glycerides contain chiefly oleic acid, and lesser quantities of stearic and palmitic acids (Skellon *et al*, 1962). Some preliminary work has been done on phenolic compounds extractable from the bark and two substances, sugiol and nimbiol have been isolated, which appear to be derived from terpenoid sources (Sengupta *et al*, 1960). The garlic odour of the seeds and oil, and the presence of sulphur (as an impurity) in solids obtained from the oil suggest the presence of compounds similar to diallyl disulphide (Siddiqui, 1942). This may in turn, suggest that

substances like "ajoene", a recently discovered substance with anti-thrombotic activity may be present (Bloch *et al*, 1986).

1.3.5.1 TRITERPENOIDS

All the well-characterised compounds identified in neem tree belong to the class of triterpenoids. From diverse biosynthetic studies in plants, these are known to be derived from acetate, via mevalonate and squalene, which is then cyclized. From the stereochemical arrangement of the methyl groups at C-10, C-13 and C-14 and the side chain at C-17, the known triterpenoids of neem are all derived from the parent tetracyclic triterpenoids, tirucallol (structure 2) with a tirucallanane structure (Arigoni *et al*, 1955).



tirucallol (2)

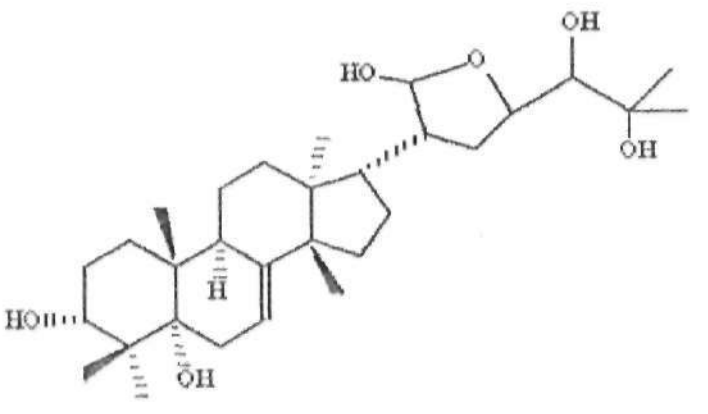
Tirucallol itself has not been isolated from any neem product but all the substances that have been characterised, can be considered as successive rearrangement and oxidation products of tirucallol. These can be divided into:

- triterpenoids with the side chain intact but in which C-24 to C-27 have been lost (as in tetranortriterpenoids), and further altered products (penta- and hexa-nortriterpenoids);

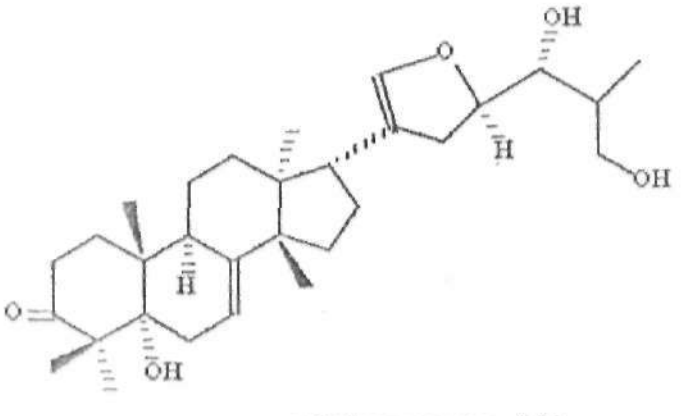
- those in which the remaining carbon atoms are cylized into a furan ring, the limonoids;
- opening of the rings or rearrangement of the skeleton, e.g. cleavage reaction involving fission of C-12 and C-13 in triterpenoids.

A. Intact Triterpenes

A number of oxidized intact triterpenes have been isolated from other species of Meliaceae. These so called protolimonoids appear to be biosynthetic precursors of the limonoids because of their biological occurrence and oxidation pattern. The only protolimonoids isolated from the neem tree are meliatriol (structure 3), isolated by Levie *et al* (1967) and nimbocenone (structure 4), recently extracted by Siddiqui *et al* (1986). The preparatory steps towards the formation of the furan ring are evident in both compounds.



meliatriol (3)



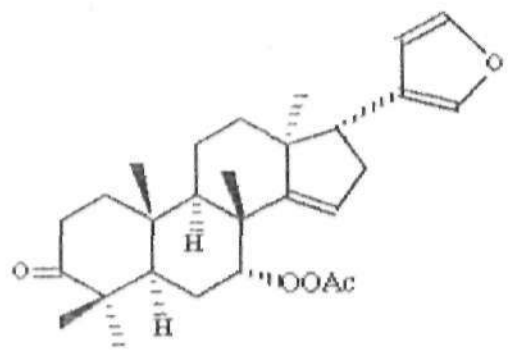
nimbocenone (4)

B. Limonoids

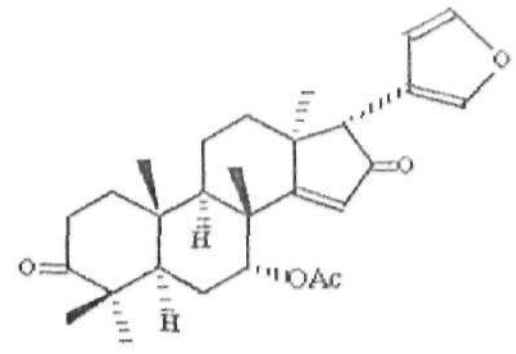
I Intact Ring -C System

Limonoids are usually defined as triterpene derivatives from which four side chain carbons atoms have been lost and the remainder have been cyclized to a furan ring, hence the alternative name, tetranortriterpenoids. As well as the changes in the side chain, they also show a rearranged carbon skeleton, with migration of the double bond to a different ring and the C-30 methyl groups from C-14 to C-8. This rearrangement has been demonstrated in vitro by the conversion of turraeanthin from *Turraeanthus africanus* into a simple limonoid.

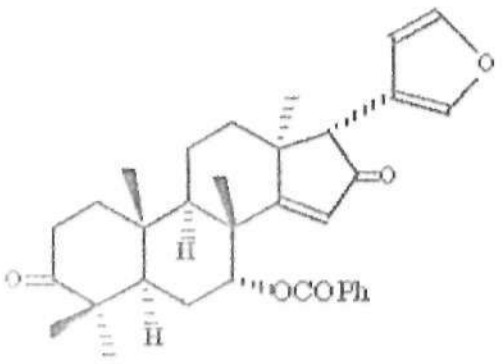
Lavie *et al* (1967, 1971) have isolated a number of simple limonoids from neem which are still closely related to the tetracyclic triterpenoids such as azadirone (structure 5) and azadiradione (structure 6) also known as nimbinin. Kraus and Cramer (1978) have isolated azadiradione 7-benzoate (structure 7) and epoxyazadiradione benzoate (structure 8).

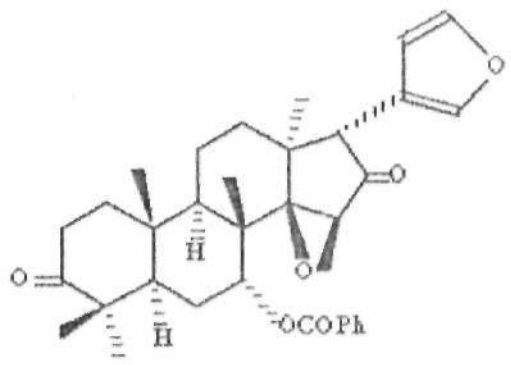


azadirone (5)



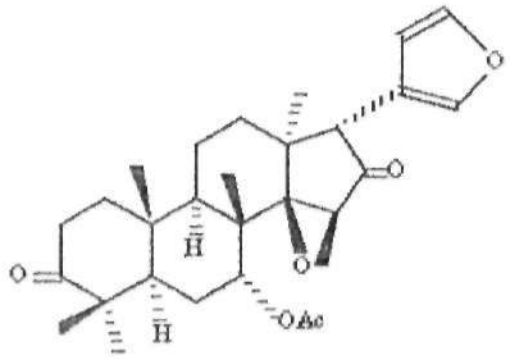
azadiradione (6)





azadiradione benzoate (7) epoxyazadiradione benzoate (8)

Nimbinin was first isolated by Siddiqui (1942) and later independently isolated by Narayanan *et al* (1967) and Conolly *et al* (1968). Lavie and Jain (1971) assigned the structure of nimbinin to epoxyazadiradione (Structure 9), which however differs in their optical rotation.

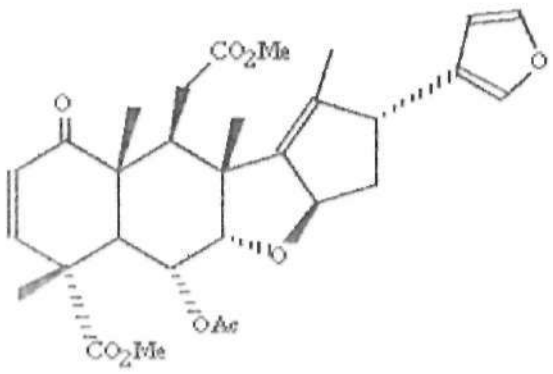


epoxyazadiradione (9)

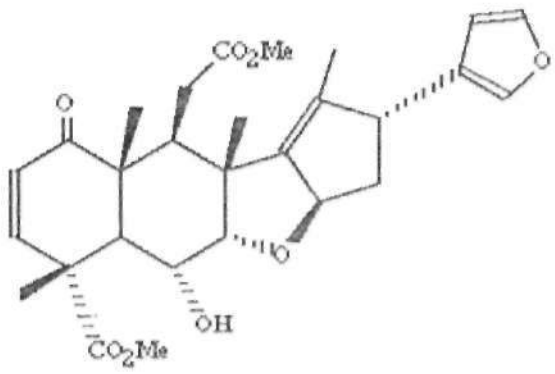
II C-Ring Opened Limonoids

The most characteristic groups of triterpene-derived compounds so far identified in neem tree are those limonoids in which ring-C has been opened by oxidation, and it is in this group of compounds that the interesting biological properties begin to appear. The first limonoid to be purified from *Azadirachta indica*, nimbin (structure 10), isolated by Siddiqui *et al* (1942) belongs to this group. It

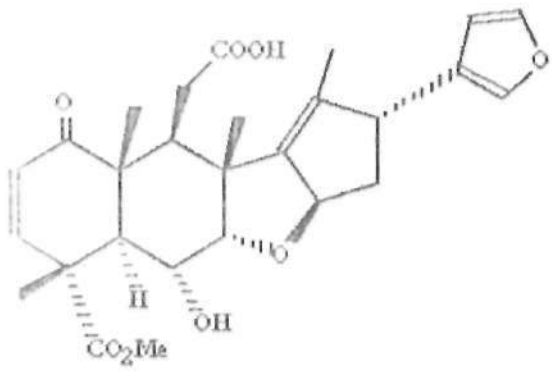
is present in many parts of the tree; isolated as a crystalline solid from seed oil, root and trunk bark. It was not until after 20 years that its structure was correctly elucidated. Its molecular formular $C_{30}H_{36}O_9$ was correctly suggested by Narasimhan (1957). Chemical studies initiated by Mitra (1956) involving the hydrolysis of the three ester moieties gave deacetylnimbin (structure 11), nimbinic acid (structure 12) and nimbic acid (structure 13). Selective hydrogenation of nimbin at C-2, C-3 double bond and sublimation of nimbic acid gave pyronimbic acid (structure 14).



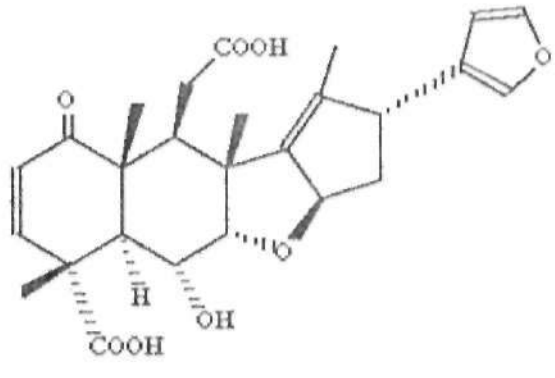
nimbin (10)



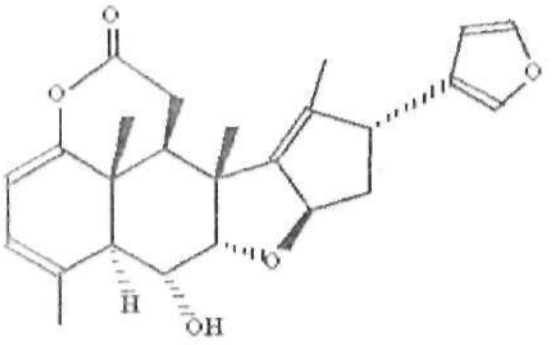
deacetylnimbin (11)



nimbinic acid (12)

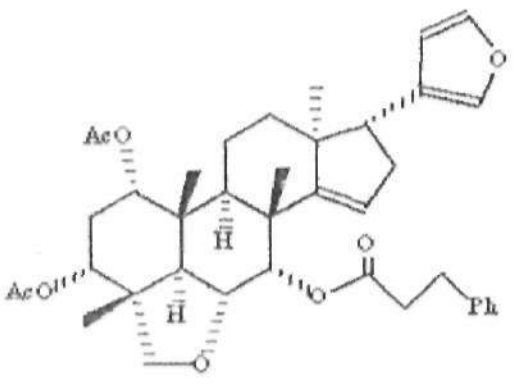


nimbic acid (13)

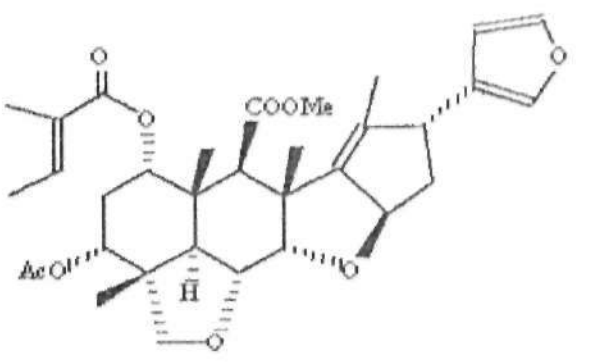


pyronimbic acid (14)

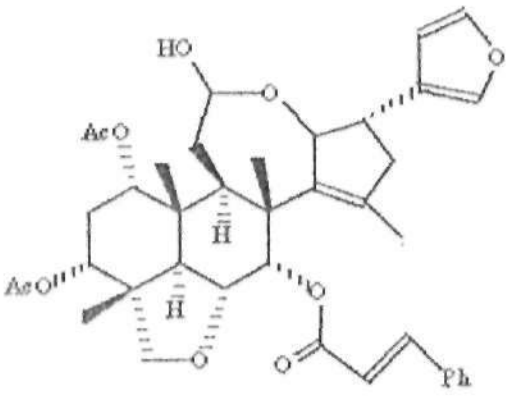
Ekong *et al* (1969) isolated two new limonoids: Nimbolin A (structure 15) and salannin (structure 16) from the trunk wood of *Azadirachta indica*. Nimbolin B (structure 17) in which the C-ring was opened was also characterised.



nimbolin A (15)

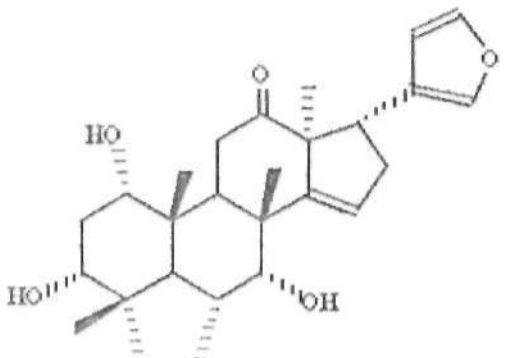


salannin (16)

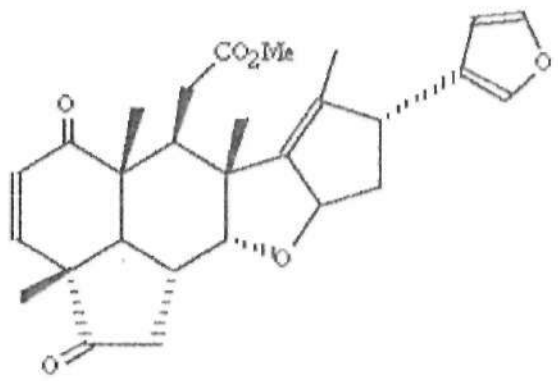


nimbolin B (17)

Mitra (1956) isolated the natural product, nimbidinin (structure 18). Many of the compounds described in this section have closely related structures, so later identification which were based on earlier elucidated structures inevitably caused some wrong structures to be proposed. An example is nimbolide (structure 19) isolated by Ekong (1967) which was later shown by NMR to be very closely related to nimbin.

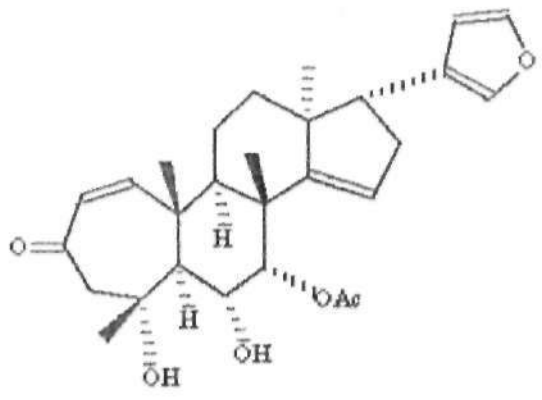


nimbidinin (18)

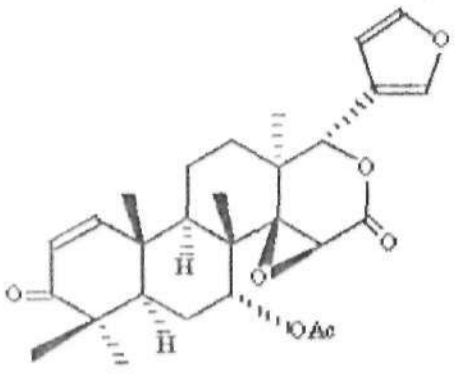


nimbolide (19)

There are also A- and D-ring opened limonoids, but these are less common in the plant parts. Examples of these are 4α, 6α-dihydroxy-A-homoazadirone (structure 20) for A-ring opened limonoid and gedunin (structure 21) for D-ring opened limonoid.



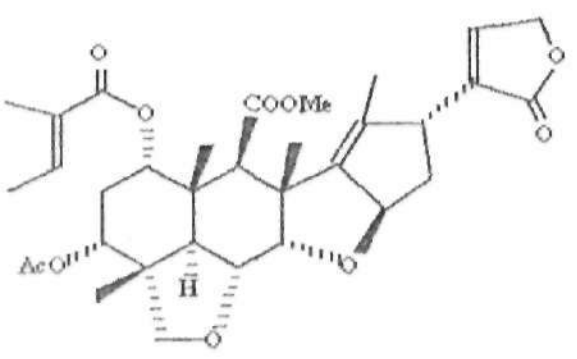
4α, 6α-dihydroxy-A-homoazadirone (20)



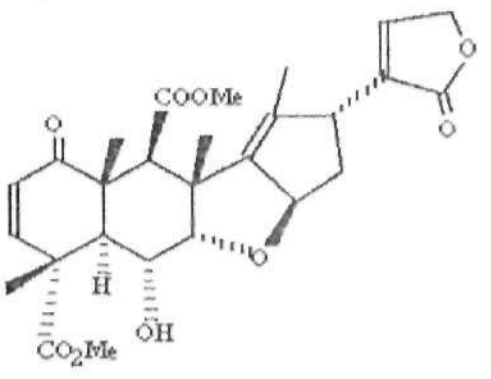
gedunin (21)

III. Products Without a Furan Ring

There are a number of examples now known which would otherwise be classed as limonoids, but which do not contain the furan ring. The first such compounds contained C-20, C-23 lactones, regarded as photo-oxidation products, possibly formed during isolation, e.g. the nimbin and salannin derivatives (structure 22 and 23), respectively.



nimbin derivative (22)



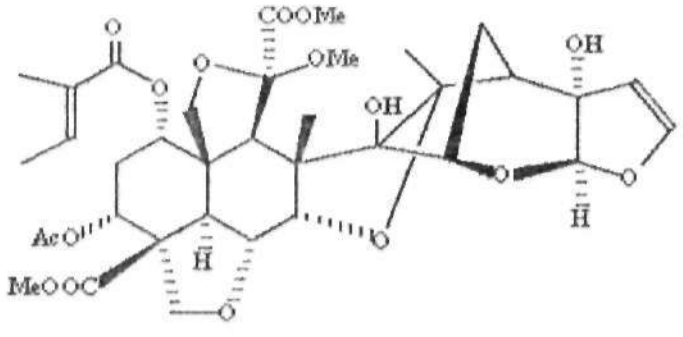
salanin derivative (23)

1.3.5.2 AZADIRACHTIN AND RELATED COMPOUNDS

Azadirachtin, from a commercial and biological point of view is the most interesting of the tetranortriterpenoids from neem tree. The molecular formular $C_{35}H_{44}O_{16}$ was determined by accurate mass measurement on a trimethylsilyl

derivative from NMR and mass spectral informations (Butterworth *et al*, 1972).

Since the correct structure of azadirachtin was published, several more azadirachtinoid structures have been isolated. Of special interest is 1-tigloyl-3-acetyl-11-methoxy -azadirachtinin (structure 24) isolated by Kraus *et al* (1987).



1-tigloyl-3-acetyl-11-methoxyazadirachtin (24)

1.4 PHARMACOLOGICAL PROPERTIES

Several pharmacological screening and evaluation studies on the neem plant and its extracts or pure isolates have been carried out. The Department of Pharmacology of King George's Medical College, Luknow, India, successfully used a lotion made from the extract of dried neem leaves to cure eczema, ringworm and scabies in clinical evaluation test (Anon, 1985). Acute cases were cured permanently in 3 to 4 days by local application of the lotion while chronic cases required about a fortnight.

Pharmacological studies have also shown that the plant extract has antipyretic (pyrogen-induced hyperpyrexia) and anti-inflammatory (rat oedema) activities (Okpanyi and Ezeukwu, 1981). When a saline solution of the sodium salt of

nimbidinic acid, a component of neem seeds was administered, urine samples showed an increased sodium chloride and potassium indicating diuretic effects (Bhide *et al*, 1958).

Nimbolide and 3-deacetyl salannin, tetranortriterpenoids isolated from the chloroform-soluble portion of an alcoholic extract of neem leaves, have shown hypotensive activity (Garg *et al*, 1985). In a cardio-vascular study, a crude extract of neem leaves injected intravenously at 30 minutes intervals, induced profound hypotension and a minimal negative chronotropic effect, which increased with increasing doses.

A 10% aqueous extract of young tender neem leaves fed to fasting rabbits at 200 mg/Kg caused a marked fall in blood glucose concentration. A maximum reduction of 27% occurred 3 hours after administration, and levels returned to normal after 12 hours. A similar hypoglycaemic effect was observed in fasted rats and to a lesser extent in guinea pigs following oral dosing (Luscombe *et al*, 1974).

Aqueous neem leaf extract caused an appreciable contraction of guinea pig ileum in tyrode's solution (Arigbabu *et al*, 1971). The leaf decoction gave a fall in the parasite count in chloroquine-sensitive strain of *Plasmodium berghei* injected in mice, and inhibited the growth of *Plasmodium falciparum* (Ekanem, 1978). Similarly, Obaseki (1985) reported that oral administration of 0.1 gm/Kg of neem extract was active against acute infection caused by *Plasmodium yoelii nigeriensis*, and increased the platelet count of treated animals. He suggested that *Azadirachta indica* should be viewed as a pointer to novel clotting, anti-inflammatory and antimalarial agents and not

as a source. In contrast, Tella (1976) observed that oral administration of aqueous extract of neem leaves to young male albino mice induced with malaria by *Plasmodium berghei*, failed to affect the course of disease in the animal.

1.5 AIMS AND OBJECTIVES

In response to the national call for "Health for All by the Year 2000" coupled with the global economic recession, the need for looking inwards to exploit the locally available medicinal plants in this country cannot be over-emphasized.

Azadirachta indica (leaves, bark and roots) is abundantly available in Nigeria and is the major source of remedy for malaria and other associated conditions for the great majority of the populace who reside in the rural areas. As results of many previous researchers have indicated, this plant can be useful in the management of malaria therapy.

One of the major problems associated with herbal remedies is standardization. The neem tree is used locally as decoction, and unspecified quantities are consumed without regard to toxicological and other adverse effects. It is therefore, the objective of this research, to explore the possibility of formulating the crude plant parts, notably the leaves and barks into tablets, so that quantification and standardization of the drug can be achieved. This will undoubtedly ensure that the plant is in a more acceptable form to the general populace, and hence improve its accessibility and availability .

To achieve this objective, the following parameters were designed to be investigated:-

- Powder characteristics of the leaves and bark. This includes particle size and size distribution, flow-ability, bulk and tapped densities, compressibility, etc.;
- Tableting properties of the crude drug powders and in combination with various drug excipients like Starch Rx1500, microcrystalline cellulose and lactose. Such properties as tablet hardness, friability, disintegration and other quality control tests will be investigated;
- Evaluation of the various methods of tablet production so that quality and acceptable tablets from the leaves and bark powders can be produced;
- Microbiological quality and properties of the powders. Microbial loads of the powders, the produced tablets and antimicrobial property of the extracts will also be assessed; and
- Pharmacological screening. This involves the evaluation of the produced tablets for their ability to reduce the degree of parasitaemia in plasmodium-infected mice when used prophylactically, curatively or suppressively. This is to enable an appropriate dosage regimen for human to be extrapolated.

It is hoped that the result of this research will shed light on the possibility of making tablets from *Azadirachta indica* so that this important medicinal plant can be presented in safer and better form for use by the populace.

CHAPTER TWO

2.0 TECHNOLOGY OF TABLET PRODUCTION

2.1 INTRODUCTION

Tablets are single solid dosage forms prepared by compression of powders, crystals or granules of drugs with or without excipients. They come in different shapes, ranging from circular, cylindrical, oval, spherical, egg or discuss-shape forms. Beside the active drug substance, they usually contain additional substances (which are in most cases, inert in nature), to ease their manufacture, disintegration and improve their physical and chemical stability.

Of the many adjuvants used in tablet manufacture, fillers occupy an important position. Fillers, also called diluents or bulking agents, are indicated especially in low dose drugs, since a minimum bulk volume is required before a powder/granule mass can be compressed into tablets of convenient size (Jaiyeoba, 1988). Although fillers are usually required to be inert, they generally tend to considerably modify the properties of the resulting tablets.

The manufacture of compressed tablets has tremendously increased following improvements and development in tableting machines.

2.2 CONSIDERATION IN THE PRODUCTION OF TABLETS

The production of a tablet is governed by a number of factors which include:

- the nature and amount of drug substance(s), its chemical and physical properties;
- the manufacturing process to be employed; and
- the method by which the tablet is to be used, i.e swallowed, chewed, dissolved in water, etc.

The stability of the drug substance is one of the most important factors to be considered in tablet pre-formulation studies. This is because the site and extent of absorption of drug in the gastro-intestinal tract is dependent on the nature of the drug. For example, if the drug is unstable in any of the fluids of the gastro intestinal tract, then some form of protection (e.g. film or enteric coating) must be afforded. Substances which would undergo appreciable hydrolysis in the conditions of wet granulation process obviously can not be made into tablets by this method. The compatibility of the drug with other tablet excipients such as binders, fillers, disintegrants, lubricants, etc, must be considered, since this will govern the choice of those additives to be used in the production of the tablets.

The dose of the drug will decide the necessity for fillers. If a filler is not indicated, there is little possibility of employing the direct compression method of tablet production especially if the active ingredient is not directly compressible. The solubility of drug, together with the proportion of drug in each tablet, will govern the need for a disintegrating agent.

The method of tablet production to be employed will be governed by the intrinsic properties of the drug substance. The process of direct compression with its savings in time,

apparatus, energy costs, and space is obviously attractive, but against this must be set higher material costs. After deciding the route of administration, the tablet is designed to satisfy the pharmacopoeial standards.

2.3 ADVANTAGES OF TABLETS AS A DOSAGE FORM

When correctly formulated and manufactured, tablets provide an accurate, uniform dosage of drug with the necessary physical and chemical properties for the required duration and intensity of therapeutic action. Modern methods are capable of large scale economic production with a high degree of tablet uniformity both within and between batches. Suitable formulations with sustained release characteristics and coatings can be produced to improve palatability or reduce the incidence of gastric irritation. Of all dosage forms, tablet presents the medicament in the highest practicable state of compaction. This permits the use of light weight and low cost containers for packaging.

2.4 BASIC COMPONENTS OF TABLET FORMULATION

The tablet does not just contain the active ingredient but also includes other substances known as excipients, with specific functions. Since the medicinally active components of most tablets are in small amounts, sometimes in milligrams, which are incompressible, there is a strong need for the addition of suitable excipients to aid the formulation and manufacture of the tablet with the desired characteristics.

2.4.1 DILUENTS

These are referred to as bulking agents. They are inert in nature and are added to the active ingredient in sufficient quantities to make a sizable tablet. Generally, a tablet should weigh at least 50 mg and therefore very low dose drugs will invariably require a diluent to bring the overall tablet weight up to this minimum. The principal diluent employed is lactose. It is relatively cheap and readily available either as coarse granular (60-80 mesh), regular (80-100 mesh) and anhydrous lactose. It is readily soluble in water, quite stable, does not react with most medicinal substances and rapidly dissociates to release its drug.

Other diluents include mannitol which is expensive; starches derived from wheat corn, and hydrolysed starch (Celutab^{R}). Starch is used in a number of official preparations not only as a filler but also as a disintegrant and binder in tablets because of its absorbent property. Calcium phosphate is a useful non-hygroscopic diluent for powder, but it should not be used as a diluent in vitamin D preparations. Microcrystalline cellulose, commercially available as Avicel^{R} is often employed as a direct compression vehicle. It is also used as a combination binder-disintegrant in the production of compressed tablets.

2.4.2 BINDERS

These are substances that act as adhesives to bind powder together in the wet granulation process. They also help to bind granules together during the process of

compression. Their use should be optimised: excess quantity results in very hard tablets that may not disintegrate on administration while very low concentration of binder results in very soft and friable tablets. Binders are used either in form of solution or in a dry form depending on the other ingredients incorporated into the formulation and the method of preparation. Commonly used binders in tablets include starch paste, ethyl cellulose (insoluble in water), methyl cellulose and gelatin. Gelatin is a protein material obtained by extraction from collagen. It swells and softens in cold water forming a clear gel on gentle heating. Depending on the binder, they are usually incorporated at concentrations ranging from 1 to 15%w/w.

2.4.3 LUBRICANTS

The lubricants enhance flow of granules, prevent adhesion of granules or powders to punches and dies and the release of tablets from its mold after compression. Lubricants act between surfaces in relative motion to prevent friction and wear. Basically, there are three categories of lubricants based on their ability to carry out certain functions:-

A. Glidants

These improve the flow properties of granules by minimizing inter-particulate friction and eliminating the problems associated with the flow of materials. Examples include talc and starch.

B. Anti-sticking Agents

They prevent sticking of granules to the faces of punches and die wall. Stearic acid and soaps are examples of the class of lubricants.

C. Proper Lubricants

These reduce inter particulate friction during compression and friction between tablet and the wall during ejection cycle, thereby facilitating tablet ejection.

The lubricants are sometimes classified according to the way in which they are incorporated into the granules. For example, external lubricants are those added immediately before compression and coat granule surface while internal lubricants are mixed with the dry powder before the addition of the granulating fluid, thus producing granules that can be described as self-lubricating (Rawlins, 1980). Examples are talc and magnesium stearate.

2.4.4 DISINTEGRANTS

These are agents added to tablets to facilitate break-up of the tablets when placed in an aqueous environment. The purpose for adding the disintegrant is to cause the tablets to break-up into smaller units so as to increase the surface area of the tablet fragments and hence promote rapid release of the drug. To do this, the disintegrant operates in a manner opposite a binder. Starch is the commonest dis-integrant in tablet formulation and is believed to act by swelling, increasing inter-particulate pores that eventually breaks the tablet into individual granules. Other dis-

integrants are modified starches, cross-linked polyvinyl pyrrolidone, cation exchange resins and cellulose materials.

2.4.5 COLOURING AGENTS, FLAVOURS AND SWEETENERS

Colouring agents improve the appearance of tablets by making it attractive and aesthetic. They are also used as manufacturing control. They are inert and soluble. Examples are amaranth and tartrazine.

Flavours and sweetening agents are important in chewable tablets, sublingual tablets and lozenges. They mask objectionable taste and odour and this influences acceptability by patients. Examples include sugars, glycerine and sorbitol. Menthol has a cooling sensation while chocolate is generally used to mask bitter taste.

2.5 POWDER TECHNOLOGY IN RELATION TO TABLET FORMULATION

Discussion of characteristic and behaviour of powders become imperative because the ingredients employed in tablet formulation (active ingredients and excipients) are usually in powder or crystalline forms. Generally, powders can be defined as a collection of discrete particles of less than 1mm in diameter (Carter, 1983). Powders are nonhomogeneous in structure consisting of different shapes and sizes with inter-particle friction and a multiple of interacting surfaces. They also have voids or air spaces between them which predisposes them to large multitude of forces like electrostatic, van der waals, etc..

The powders therefore, show complex physico-chemical and mechanical properties. They sometimes show similarities to liquids in that they deform and flow when stressed and to

solids in that they exhibit both elastic recovery and brittle fracture (Rawlins, 1977).

Unlike conventional solid, powder becomes appreciably compressed by the application of pressure. It is this property that is employed in tableting. Emphasis is placed on the the ability of the powders to be compressed into tablets and its subsequent performance during drug delivery. Some of these unique properties of powders are:

(i) Particle Size

Various chemicals and physical properties of drug substances are affected by their particle size distribution and shapes. The effect is not only on the physical properties of solid drugs but also in some instances, on their biopharmaceutical behaviour.

The particle size of pharmaceutical powders play a role in the tablet homogeneity. Fine materials are distributed uniformly but flow less as compared with relatively coarse ones. Particle size is also a factor in the stability of certain materials. Fine materials are relatively more open to attack from atmospheric oxygen and humidity than coarse materials.

It is therefore, important to narrow the difference in particle size of the various components when mixing powders so as to achieve a better mix.

(ii) Adhesive/Cohesive Properties

The attraction is usually ascribed to non-specific Van der Waals forces although in most materials, moisture layer can confer cohesiveness by action of surface tension at points of contact (asperites). For these reasons, an

increase in humidity can lead to sudden increase in cohesiveness and complete loss of mobility in a powder material which ceased to flow. Cohesion and adhesion increase as the particle size decreases and as the number of points of contact (asperites) in a given area of apparent contact increases.

(iii) Flow Properties

The flow property of powders is extremely complex and is influenced by many factors. The accuracy of weight and dose depends on the regularity of the flow during tableting. Materials that will pass through an orifice can be conveniently labelled as free-flowing while material which will not, even though its particles are very much smaller than the orifice, can be labelled as cohesive.

The following are techniques used to characterise the flow properties of powders;-

(a) Angle of Repose Determination

This is the angle of elevation to the horizontal at which the powder begins to slide upon itself. The type of flow ascribed to each angle is indicated below:-

Degree	Type of Flow
< 25	Excellent
25-30	Good
30-40	Possibility
> 40	very poor

CARR'S Index

The Carr'3 index is expressed as:

$$\frac{\text{(Tapped density - Bulk density)}}{\text{Tapped density}} * 100$$

The Carr's index indirectly measures the ability of the powders to rearrange and pack neatly by reducing the degree of porosity within the packed ma33. The lower the value of the Carr's index, the better is the flow properties of the powders.

Compression or Deformation Characteristics

The behaviour of powders under compression is significant in pharmaceutical tableting. All materials change shape and/or volume when subjected to external stress. If the change i3 spontaneously reversible on removal of the force, then the deformation is said to be elastic e.g paracetamol powder. But when the change is irreversible on removal of force, then the deformation is said to be plastic or fracture e.g aluminium hydroxide. The powder properties play a major role in, the consolidation and properties of the compressed tablets.

1IETH0DS OF TABLET PRODUCTION

The basic tableting steps include weighing of active ingredients, diluent and endo-disintegrants where applicable and dry-mixed. The binder is then subsequently added to the dry-mix by the dry or wet method.

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2.6.1 WET GRANULATION

The most widely used and most general method of tablet preparation is the wet granulation method. Its popularity is due to the increased probability that the granules will meet all the physical requirements for the compression of good tablets. Its major disadvantages include increased processing time and higher number of steps involved in the process. The steps in the wet granulation method are:

1. weighing;
2. mixing;
3. granulation;
4. screening the damp mass;
5. drying;
6. dry screening;
7. lubrication; and
8. compression.

Essentially, the active ingredients, diluents and disintegrants are mixed or blended well. The powder blend may be sifted through a screen of suitable fineness to remove or break-up lumps.

Solutions of the binding agent are added to the mixed powders with stirring. The powder mass is wetted with the binder solution until the mass has the consistency of damp snow (Clarkson, 1950). If the granulation is over-wetted, the granules will be hard, requiring considerable pressure to form tablets which may have mottled appearance. If however, the powder mixture is not wetted sufficiently, the resulting granules will be too soft and cause difficulty during compression.

The wet granulation is forced through a screen. Small batches can be manually screened. The granulation is then spread on trays and dried in conventional ovens or in fluid-ized-bed dryer. The dried granules is subsequently size-reduced by passing it through smaller mesh screens. The equipment involved depends on the quantity or size of the batch.

2.6.2 DRY GRANULATION METHOD

When the tablet ingredients are sensitive to moisture or are unable to withstand elevated temperatures during drying but have inherent binding and cohesive properties, slugging may be used to form granules. In this process, the powdered materials are compressed into large sheets or oversize tablets (slugs) which is then broken down to form granules of required size. It is important that either the active ingredient or the diluents possess cohesive properties. Aspirin is an example of material whose granules can be produced by this process. List-wise, the steps involved are:

1. weighing
2. mixing
3. slugging
4. dry-screening
5. lubrication, and
6. compression.

2.6.3 DIRECT COMPRESSION

As the name implies, direct compression simply consist of compressing tablets directly from powdered materials without modifying the physical nature of the materials

itself. Materials like potassium salts (chlorate, bromide) and ammonium chloride possess cohesive and good flow properties, attributes that enhance their direct compressibility. It is essential that where active ingredients constitute the major part of the tablet, they should in themselves possess these two characteristics for it to be directly compressible. In case of low dose drugs, direct compressible excipients are usually incorporated to aid tablet production by this process. The gradual improvement of formulation additives and development of mechanical feeding devices for the high speed rotatory tableting machines has made direct compression the preferred method for tablet production. Of all the methods, direct compression is the most adaptable to automation.

Direct compression method should produce tablets of faster dissolution rate since no colloidal binders such as gelatin or starch paste are used to surround the granules.

7 TABLETING MACHINES

There are basically two types of tableting machines that are in common use. Tablets may be compressed in single punch eccentric machines which have an output of 60 -90 tablets per minute or on multi-station tablet machines exemplified by rotary tablet press which produces up to 50,000 tablets per minute. With development in machinery, sophisticated tableting machines that can produce as much as 500,000 tablets per minute are now available.

2.7.1 SINGLE PUNCH MACHINES

Single punch tablet press are only indicated in small scale and pilot scale production. They can be driven either manually or electrically. The machine consists of a frame containing a horizontal plate into which the die is fitted. Attached to this plate is the feed shoe containing the granules to be compressed.

The lower punch assembly has two collars on it. The bottom collar regulates the depth to which the lower punch descends during a compression cycle, which in turn controls the volume and therefore the weight of granules to be compressed. The upper collar allows the punch to be adjusted so that at the top of the compression stroke, which is at the point of ejection of the tablet, it is level with the upper surface of the die.

The upper punch is also fitted with a collar which adjusts the depth to which the punch descends into the die. By this means, the compression pressure can be adjusted as required. A compression cycle in a single punch machine involves the following steps:

- the feed-shoe moves over the die-cavity,
- the lower punch descends to a preset level,
- granules flow from the hopper into the feed-shoe, and fills the die-cavity,
- the upper punch descends into the die-cavity and compresses the granules,
- the upper punch ascends, and
- the lower punch also ascends and ejects the tablet from the die-cavity.

2.7.2 ROTARY TABLET PRESS

The rotary machine consists of tablet hopper, a feed frame and a revolving turrette containing at least sixteen sets of punches and dies. They are the commonest tablet press in the industries because of their large output.

In this machine, the granules flow from the hopper and fills the feed frame. As the dies pass in turn under the feed frame, the lower punches fall, drawing the granules into the die. The extent to which the lower punches fall is controlled so that the tablet weight can be controlled. The dies then pass under a scrapper which removes excess granules. Compression is achieved by both sets of punches moving into the die cavity over the rollers. This action squeezes the granules and forms the tablets. Both sets of punches simultaneously rise and eject the tablet, which is then removed by a scrapper.

2.8 QUALITY CONTROL PARAMETERS OF TABLETS

The control of tableting materials and processes is essential to ensure that the products are of high quality. The quality control tests carried out on tablets can be broadly classified into two namely:

A PHARMACOPOEIAL TESTS

They are tests that deal with standards that ensure compliance with pharmacopoeial specifications. These include:

(i) Uniformity of Weights Test

This is to check if tablets produced using the same formulation and under identical production parameters contain the proper amount of drugs. Individual tablets are usually weighed and their percentage deviation from the mean is calculated. The permitted deviation limits are given below.

Average Weight of Tablet	Percentage Deviation
80 mg or less	10
more than 80 mg but less than 250 mg	7.5
250 mg or more	5.0

Not more than 2 tablets out of 20 are permitted to have percentage deviations greater than stated above and no tablet should have a percentage deviation that is twice the limit.

(ii) Test for Content of Active Ingredient

This test essentially ensures that the tablets contain the stated amount of active ingredient. In this test, 20 tablets are taken at random, powdered and a chemical assay is performed on aliquots of the powder. This test in actual sense, determines the average content of the active ingredient since a pool of 20 tablets are used in the analysis. This test does not determine the activity or bio-availability of the active ingredient.

(iii) Test for Uniformity of Content

This guards against variability in drug content within a sample of tablets. Usually, 10 tablets are taken and each individual active ingredient content is assayed and then compared to specified standards. Nine out of the ten tablets are required to fall within a specified limit.

(iv) Tablet Disintegration Test

It is generally accepted that before a drug can be available for absorption into the body, it has to be in solution. Disintegration and dissolution may be rate determining steps in the absorption of drug forms. However, fast disintegration time of a dosage form does not necessarily mean fast absorption of a drug. In this test, the tablets are agitated in an aqueous medium thermostated to the body's temperature and the end point is reasonably non-subjective. All uncoated tablets are required to disintegrate within 15 minutes while coated tablets should disintegrate within 60 minutes in water or 3 minutes in a disintegrating medium of 0.1M HCl.

(v) Dissolution Test

Drug absorption and physiological availability are largely dependent upon having drug in the dissolved state. Therefore, dissolution is an important process which a drug in a solid dosage form must undergo when administered before it can elicit its pharmacological and or therapeutic action. The tablet to be tested is normally put in a basket and immersed in a defined dissolution medium. At time intervals or after 45 minutes, samples of the dissolution medium is

withdrawn and analysed for the percentage content of the active ingredient already in solution. It is expected that by 45 minutes, 70% of the drug should have been dissolved.

B. NON-PHARMACOPOIEAL TESTS

The most important tests under this category involve the measurement of the ability of the tablet to retain its physical integrity. They are:-

(i) Tablet Hardness

Hardness implies that the tablet possesses a surface which is resistant to penetration. Hardness is often associated with the ability of the tablet to withstand excessive pressure. Hardness is usually determined by subjecting the tablet to increasing compression load until it breaks. Common instruments employed in determination of this parameter are the Monsanto, Erweka and Strong-Cobbs hardness testers.

(ii) Friability

This is an indicator of the tablet's resistance to abrasion. This is because the tablets are likely to be subjected to a tumbling motion during packaging and transportation, which may abrade small particles from the tablet's surface. The Roche friabilator is probably the most commonly used abrasion tester. The weight loss during abrasion is calculated and should not be more than 0.8%.

(iii) Thickness

This is another non-pharmacopoieal test. The thickness of tablets is related to the degree of compaction of the

material used for the formulation. Variation in thickness values often indicate problems in the flow of the granules into the die-cavity. The most common instrument used for measurement of tablet thickness is the vernier caliper.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals

Buffer tablets, BDH Chemicals Ltd, Poole, England, U.K.

Chloroform, BDH Chemicals Ltd, Poole, England.

Ethyl alcohol, BDH Chemicals Ltd, Poole, England.

Giemsa's stain solution, May and Baker, Germany.

Glycerin, BDH Chemicals Ltd, Poole, England.

Lactose. May and Baker, Germany.

Magnesium stearate,. Hopkin and Williams, U.K.

Maize starch, May and Baker, Germany.

Grower's Marsh (Animal feed), Sanders Feeds, Kaduna, Nigeria.

Methanol, BDH Chemicals Ltd, Poole, England.

Methyl cellulose, BDH Chemicals Ltd, Poole, England.

Microcrystalline cellulose (Avicel^{R}), Honey Well and Stein Ltd, U.K.

Polyvinyl pyrrolidone (PVP), BDH Chemicals Ltd, Poole, England.

Sodium chloride, May and Baker, Germany.

Starch R_x 1500, Colorcon Ltd, U.K.

3.1.2 Microbiological Media

Deoxycholate citrate agar, Oxoid Ltd, Basingstoke, Hants, England.

MacConkey agar, Becton Dickinson Microbiology Systems, Cockeysville, USA.

Mannitol salt-phenol red agar, E. Merck, Darmstadt, Germany.

Nutrient agar, E. Merck, Darmstadt, Germany.

Pseudosel agar, Becton Dickinson Microbiology Systems, Cockeysville, USA.

Sabouraud dextrose agar, Oxoid Ltd, Basingstoke, Hants, England.

3.1.3 Microbiological Specimen

3.1.3.1 Microorganisms

Bacillus subtilis, NCTC 8236

Candida albicans, clinical isolate from Microbiology department, Ahmadu Bello University, Zaria.

Escherichia coli, NCTC 10418

Pseudomonas aeruginosa, NCTC 6750

Staphylococcus aureus, NCTC 6571

3.1.3.2 Malaria Parasite

Plasmodium yoelii nigeriensis (N67 Chloroquine-sensitive strain), obtained from the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria.

3.1.4 Botanical Material

Azadirachta indica leaves and bark, obtained within the premises of Ahmadu Bello University, Zaria.

3.1.5 Animal Specimen

Swiss albino mice, initially bred in the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

3.1.6 Equipment and Instruments

Test Sieve Shaker, No. 7416, Endecotts Ltd, London, England.

Mettler Top Loading balance, No. HS 00531

Friabilator, Erweka TA-3R, No. 42961

Tablet Disintegrating Apparatus, Erweka Type ZT3, No. 41728

Single punch tableting Machine, Erweka AR 400, No. 41831

Water-bath (Gallenkamp), No. N-BJE-800-0106

Hot plate (Gallenkamp), No. HL052

Mortar and Pestle (wood type)

Hot air Oven (Gallenkamp), Size 3 BS, 2648

Vernier caliper (Gallenkamp)

Flowameter, Erweka TA-3R, Type GDT, No. 42232

Optical Microscope, Reichert Scientific Instruments, USA

Incubator, Baird and Tatlock Ltd, Essex, U.K.

Bench Autoclave, Adelphi Man. Co. Ltd., Birmingham, U.K.

Colony counter (Gallenkamp), No. N-CNW-300-010NP

Light Microscope, Wild M11, Switzerland.

Monsanto Hardness tester

Liquid dispenser, Gilson, France.

3.1.7 Glasswares

Petri dishes

Glass slides

Cover slips

Test-tubes

Pipettes, 5 and 10 ml sizes.

Squared graticule, Graticules Ltd, Tonbridge, Kent, England.

3.1.8 Miscellaneous

Stop watch, type C20S

Retort stand

Plastic cages of size 29 cm x 11 cm x 12 cm)

Pair of scissors

Dispensing bottles

Forceps

Staining jar, B31740/1

Tally counter, N-CTL-340-Y

Brushes

Wood shavings

Test-tube racks

Cork borer No. 4

3.2 EXPERIMENTAL

3.2.1 Collection and Authentication of Neem Leaves and Barks

The leaf and bark of *Azadirachta indica* were collected within the premises of Ahmadu Bello University, Samaru, Zaria. The plant parts were authenticated in the Department of Pharmacognosy and Drug Development of the Ahmadu Bello University, Zaria. Herbarium specimens of the plant parts were prepared in the Department of Biological Sciences, of the same University.

The fresh leaf and bark were first allowed to dry in the open air and further dried in a Gallenkamp hot air Oven at 40°C for 6 hours in case of the leaves and 12 hours in case of the barks. The leaves and bark were thereafter ground in a wooden mortar and pestle.

3.2.2 Particle Size Classification of the Crude Powder

The leaf and the bark powders were separately placed n a stack of three sieves of descending mesh sizes of 500 µm,

250 µm and 150 µm. The sieves were vibrated on an Endecott test sieve shaker for 15 minutes after which powders of sizes between 250 µm and 150 µm were collected and used for the experiment. A powder mix of bark and leaf were similarly categorised.

3.2.3 Determination of Angle of Repose

The angle of repose, often defined as the maximum angle possible between the surface of a pile of powder and the horizontal plane, was determined as follows:-

A glass funnel was placed on a retort stand at a height of 12 cm from the horizontal surface. A cardboard paper was then placed to cover the hole of the funnel at the lower end so that it fitted tightly but loose enough to be moved away. 10 gm of the test powder was poured into the funnel. The cardboard paper was removed and with the aid of a stop watch, the time it took the whole powder to flow through the funnel was measured. The funnel was then lowered to touch the tip of the powder heap and using a ruler, the height of the tip measured. The base (contour) of the powder was traced and the radius of the circle determined. From these data, the tangent of the various angles formed by the powders were computed.

3.2.4 Determination of Carr's Index

25 gm of the powdered sample (leaf, bark or their binary mixtures) was gently poured into a 100 ml measuring cylinder and the initial volume of the powder was recorded. The cylinder was then tapped 300 times on a soft surface, at tapping rate of 30 taps per minutes from a height of 3 cm.

Thereafter, the new volume (tapped volume) was also recorded.

The bulk and tapped densities were calculated using the below formulae:

$$\text{Bulk density} = \frac{\text{Weight}}{\text{Bulk volume}} \quad \text{.....3.1}$$

$$\text{Tapped density} = \frac{\text{Weight}}{\text{Tapped volume}} \quad \text{.....3.2}$$

Data obtained from the two densities are used in calculating Carr's index using the following equation:

$$\text{Carr's index} = \frac{\text{Tapped density - Bulk density}}{\text{Tapped density}} \text{ X } 100\%$$

For each powder, three sets of readings were taken.

3.2.4 Determination of Flowability

50 gm of each powder was poured into the funnel of the Erweka TA-3R flowameter. The apparatus was started and the time it took the powder to flow through the orifice was noted. The flow rate which is the amount of powder that passes through the orifice in one second was determined. For each powder, three sets of readings were taken and the average recorded.

3.2.6 Determination of Moisture Content

5 gm each of the leaf and bark powders were taken and placed on a previously weighed piece of paper. They were dried in a Gallenkamp hot air oven at 50-60 °C. The powders were weighed periodically until the weights were constant. The moisture contents, which is the percentage fraction of the loss in weight to the initial weight was then computed.

3.2.7 Particle Size Distribution

The optical microscope method was employed in this determination. The eye piece of Wild M11 light microscope was fitted with a graticule. The dimensions in the eye piece were then calibrated using a standard micrometer. From the calibration, 100 units on eye piece was equivalent to 1000 µm.

A random mix of each powder was taken and mounted on a glass slide using glycerin as the mountant. The sizes of the images were determined by correcting them with the calibration on the eye piece. The sizes were recorded for 300 particles. The percentage frequency within various size ranges was calculated from which plots of cumulative percent sizes were drawn.

3.2.8 Tablet Production

Two tablet production methods were employed in this study namely:-

3.2.8.1 Direct Compression

The leaf and bark powders and their admixtures with lactose, Starch R_X 1500 and Avicel^R (as diluents) after proper blending were directly compressed on an Erweka AR 400 single punch tableting machine, fitted with 8.5 mm diameter set of punches and dies. The volume of the die-cavity was adjusted to accommodate 300 ± 10 mg of the powders for compression into tablets. Forced feeding of the die-cavity was resulted to, due to the very low batch size.

Concentrations of diluents in the admixture varied from 25% to 100%. Tablets were compressed at pressure

setting of 7.5 KN, which was varied only in exceptional cases e.g. when tablets could not be formed at this pressure.

3.2.8.2 Wet Granulation

The composition of the working formula employed in the production of tablets by the wet granulation method is given below..

Powdered plant part	40.0 gm
Maize starch (as disintegrant)	4.0 gm
Binder solution	q.s
Magnesium stearate (lubricant)	0.2% w/w of dry granules.
Each Batch contains 147 tablets,	each of 272 mg drug content.

The crude drug was manually mixed with the maize starch using a spatula prior to the incorporation of the binder solution.

Preparation of Binders

(i) Maize starch mucilage

5 gm of maize starch was weighed into a calibrated beaker. 25 ml of cold distiled water was added to the beaker and stirred until a homogeneous suspension was obtained. 60 ml of distiled water was placed on a hot plate and boiled. The starch suspension was added to the hot water until a translucent jelly mass was formed. The weight was made up to 100 gm with hot distiled water (to give a 5% w/w mucilage)

and allowed to cool to about 40°C before it was incorporated as a binder.

(ii) Polyvinyl pyrrolidone Mucilage

5% w/w mucilage of polyvinyl pyrrolidone was prepared in the same manner as described for maize starch.

(iii) Methyl cellulose Mucilage

5 gm of the powder was weighed and hydrated in cold water (about a $^1/_3$ of the total water required) and heated to boiling. It was allowed to stand for 30 minutes. The rest of the water was added as ice-cold and the mixture stirred until homogeneous.

Wet granulation was manually carried out in a large pan. Aliquots of the binder solution that had cooled to about 40°C was added to the mixture of the powdered drug materials until a sufficiently wet mass (i.e. when there is a feeling of dampness and consistency of the granules) was formed. In each of the batches, 80% of the binder solution was found to produce good wet mass. This concentration was maintained through all the wet granulation processes. The wet mass was passed through a 1 mm screen mesh to produce wet granules. These were dried in a Gallenkamp hot air oven at 40°C for 45 minutes with constant turning. The dried granules were passed through another 1 mm screen to break aggregates before being subjected to a second drying period to obtain a constant weight granules.

The granules obtained were separated into various size fractions as described in 3.2.2. 0.2% w/w of fractionated magnesium stearate powder was levigated into the dried

granules and compressed into tablets in an Erweka AR 400 single punch tableting machine as earlier described for direct compression process in 3.2.8.1.

A 24 hour period was allowed for all batches of tablets produced to undergo stress relaxation before subjecting them to quality control tests.

3.2.9 QUALITY CONTROL TESTS

The quality control parameters that were investigated include:- average weight, weight variation, thickness, disintegration, hardness and friability.

3.2.9.1 Average Weight

Twenty (20) tablets of each batch were randomly selected and weighed on a Mettler top loading balance and the average weight of a tablet was determined.

3.2.9.2 Weight Variation

Twenty (20) tablets were randomly taken from each batch and weighed together. Thereafter, each tablet was individually weighed. From the mean, the percentage weight variation of each tablet was calculated.

3.2.9.3 Tablet Thickness

Ten (10) tablets were taken at random from every batch and their thickness measured with a vernier caliper. The average thickness of tablets from each batch was then computed.

3.2.9.4 Disintegration Test

The disintegration times of the produced tablets were determined according to the BP procedure. Erweka disintegration tester, type ZT3 was used in this test. Distiled water thermostatically maintained at 37°C was used as the disintegration medium. Six tablets were placed in the tube, of which the lower end was fitted with a gauze disc made of rust-proof wire. The disintegration apparatus was calibrated to operate at thirty cycles per minute. A cycle is made up of one complete up and down movement. The apparatus was adjusted such that at the highest up movement, the tube containing the tablets is completely out of the disintegration medium and completely immersed at the lowest down movement. For each batch of tablets, the experiment was repeated to yield two sets of readings.

3.2.9.5 Hardness Test

The Monsanto hardness tester was used in measuring the crushing strength of the tablets. A tablet was held between a fixed anvil and a moving jaw. The load was gradually increased until the tablet just fractures. The value of the load at this point gives a measure of the tablet hardness in KgF. For each batch, the hardness of ten (10) tablets were determined from which the average was computed.

3.2.9.6 Friability Test

Twenty (20) tablets were randomly selected and brushed lightly to remove any powder from the surface and then weighed on a Mettler top loading balance. Thereafter, they

were placed in Erweka TA-3R friabilator for subjection to abrasion. The friabilator was allowed to operate for 4 minutes at a speed of 25 rpm. After the 100 rotations, the tablets were taken out, dusted and re-weighed. From the two weight values, friability for each batch of tablet was then computed as shown below:

$$\text{Friability (\%)} = \frac{\text{Weight loss}}{\text{Original weight}} \text{ X } 100$$

3.2.10 Microbiological Studies on Neem Tree

3.2.10.1 Determination of Microbial Contamination Levels and Flora

Nutrient and Sabouraud Dextrose agars (for bacterial and fungal organisms respectively), were reconstituted in distiled water according to the manufacturer's directives. They were dispensed in 20 ml quantities into bottles and sterilized at a temperature of 121°C and pressure of 15 psi for 15 minutes in Adelphi Bench Autoclave. Before use, the sterilized agars were melted and allowed to cool to 40°C.

Tablets were first of all, crushed into powders in sterile mortar and pestle. 1 gm of the powders (leaf, barks and tablets) was suspended in 9 ml sterile distiled water to give a 1 in 10 dilution. From this homogenate suspension, 1 ml was withdrawn and added to another 9 ml of sterile distilled water to produce a 1 in 100 dilution.

The pour plate method was employed in the enumeration of microbial contamination level. 1 ml each of the 1:10 and 1:100 homogenate suspensions were mixed with the previously melted and cooled agars. This was poured into sterile petri-dishes, allowed to set and then incubated at 37°C for 24-48

hours in case of the nutrient agar or 22°C for 3-5 days in case of Sabouraud dextrose agar. Each dilution level was performed in duplicate. Colonies growing in and on the agar were counted with the aid of a Gallenkamp Colony Counter.

Preliminary identification of the bacteria contaminants were based on their colonial and cell morphology in conjunction to Gram staining as described by Mackie and McCartney (1989). Identification of fungal organisms were also primarily based on their morphological characteristics under the microscope as recommended by Mackie and McCartney (1989).

3.2.10.2 Test for Determination of the Presence of Specific Organisms

This test is geared towards the detection of some pathogenic and indicator organisms, namely. *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* and *Staphylococcus aureus*, which are not allowed to be present in such samples.

i. **Test for Presence of *Escherichia coli***

For the detection of Escherichia coli, MacConkey agar was used as the selective and diagnostic medium. The powder was reconstituted in accordance with manufacturer's directive and prepared in a a similar manner as described for Nutrient agar (3.2.10.1). 1 gm of the powders was suspended in 9 ml of sterile distiled water. From this homogenate suspension, 1 ml was withdrawn and mixed with 20 ml of the melted and cooled MacConkey agar and poured into sterile plate. Duplicate plates were prepared for each sample. They were incubated at 37°C for 1-2 days in a Baird

and Tatlock incubator. Colonies that might exhibit red colouration with red precipitation zones were taken for further identification.

ii. **Test for Presence of *Pseudomonas aeruginosa***

The same procedure as described for *Escherichia coli* was followed in the detection of this organism. However, in the place of MacConkey agar medium, Pseudosel agar was used as the selective and diagnostic medium. Attention was paid to greenish or greenish blue or yellowish green colonies with greenish diffusible pigment in case further identification process would be needed.

iii. **Test for Presence of *Salmonella* spp**

The same procedure described for *Escherichia coli* was adopted in the detection of *Salmonella* organisms. The selective and diagnostic medium used was however, deoxycholate citrate agar. Presumptive presence of these organisms would be indicated by colonies exhibiting black or brownish black colouration with metallic sheen.

iv. **Test for Presence of *Staphylococcus aureus***

For the detection of *Staphylococcus aureus,* Mannitol salt and phenol-red agar was used as the diagnostic and selective medium. The same procedure as described for *E. coli* indicator was employed. Attention was paid to yellowish or bright orange colonies with yellow surrounding which on microscopy are gram positive cocci in clusters.

3.2.10.3 Determination of Antimicrobial Properties of Extracts of Neem Tree

i. Preparation of Extracts

Aqueous and ethanolic extracts were prepared as described below and used in the evaluation of antimicrobial property.

For the aqueous extract, 5 gm of the powders (leaf and bark) was added to 100 ml of distiled water in a beaker. It was heated to boiling on a hot plate with continuous stirring for 30 minutes. The supernatant which contained the extract was decanted and dried in a crucible over a water-bath. From the dried extract, 1 and 10 % w/v aqueous suspensions were prepared.

In the ethanol extraction, 5 gm of the powder was also placed in a beaker containing 100 ml of ethanol (98%). It was tightly covered and intermitently shaken for six hours and then left overnight. Thereafter, the supernatant was decanted and preliminarily dried over a water-bath. Further drying was achieved in an oven at 40°C for a few minutes. 1 and 10 %w/v suspensions in water were prepared as follows: the weighed amounts of the ethanolic extracts were dissolved in minimal amount of alcohol and the volume made up with water.

ii. Preparation of Inoculum

- The following strains of microorganisms were used:-

Bacillus subtilis NCTC 8236

Candida albicans - clinical isolate obtained from the Department of Microbiology, A.B.U., Zaria.

Escherichia coli NCTC 10418

Pseudomonas aeruginosa NCTC 6750

Staphylococcus aureus NCTC 6571

A single colony from an overnight growth was suspended in sterile normal saline and a dilution of 1:100 made to give an adjusted concentration of 10^7 cfu/ml (National Committee for Clinical Laboratory Standards, 1990). 0.2 ml of the standardized suspension of the test organism was added to 20 ml of previously melted and cooled Nutrient agar, properly mixed and poured on sterile petri-dish. It was allowed to set at room temperature. Thereafter, six holes were bored equidistant to each other using cork-borer No 4. The base of each hole was sealed with 1-2 drops of molten agar.

Ten (10) drops of each concentration of the crude extract was added to each hole. For each concentration, triplicate holes were filled. In each case, controls were applied to assess the role of the diluent used. Diffusion time of 2 hours at room temperature was allowed before incubation of the plates at 37°C in an incubator for 24 hours. The diameter of zones of inhibition of each extract for each test organism was measured with a ruler.

3.2.11 Determination of Antimalarial Activity of Extract of the Neem Tablets

3.2.11.1 Test Animals

Swiss albino mice weighing between 18 and 24 gm were obtained from the Animal house of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Some were also bred in the Animal house of the Faculty of Pharmaceutical

Sciences, A.B.U., Zaria. They were kept in groups of five in plastic cages of sizes 29 cm x 11 cm x 12 cm. Cage beddings were made of wood shavings. The animals were fed on grower marsh (Sanders Feed, Kaduna) and generally supplied with water. Same environmental conditions were maintained for all the animals throughout the period of investigation.

3.2.11.2 Preparation of Drug Solutions

Aqueous extracts from 20 tablets were obtained as previously described (3.2.10.3). A stock solution of 40 mg/ml of each extract was prepared using 10% sugar solution (to mask the bitter taste of the extract) as the solvent. Until ready for use, stock solutions were kept refrigerated.

3.2.11.3 Preparation of Normal Saline

Sodium chloride (4.5 gm) was dissolved in 500 ml distiled water. The resulting solution was sterilized by autoclaving at 115°C and 10 psi for 30 minutes.

3.2.11.4 Preparation of Inoculum of Parasite

The N67 chloroquine-sensitive strain of *Plasmodium yoelii nigeriensis* obtained from the Department of Veterinary Parasitology and Entomology, A.B.U., Zaria, was used. Before use, the pulled infected blood was stored in liquid nitrogen at -196°C.

Five mice were used for passaging the parasite. The stored parasitized blood in the liquid nitrogen was removed and allowed to thaw. The contents were injected into five donor mice intra-peritoneally and left for 3-5 days. Once

parasitaemia has developed to 20-30% in the mice, blood was taken for further passaging.

To obtain blood from the donor mouse, it was anaesthetized with chloroform. With the aid of dissecting forceps and a pair of scissors, the chest was opened and blood obtained by cardiac puncture, which was collected using sterile syringe. This was immediately transferred into a heparinized tube and shakened. The blood was then suitably diluted with sterile normal saline so that the final inoculum (0.2 ml) for each mouse would contain 10^7 parasitized red cells (Iwalewa *et al*, 1992).

3.2.11.5 Calculation of Inoculum Size

The amount of blood to be taken from a donor for inoculation into other mice was calculated using the approaches of Jacob *et al* (1963), Peters (1980) and Olise (1984).

Jacob *et al* (1963) and Peters (1980) recommended the use of donor mice with average 38.5% parasitaemia. Olise (1984) found that 1 ml of an undiluted mice blood contain 4.62×10^9 RBC (PRBC)

It then follows that a 1 ml of blood from a donor mouse will contain: $\frac{38.5}{100} \times 4.62 \times 10^9$ parasitized RBC.

Since 10^7 parasitized RBC is required in 0.2 ml of blood, it follows that 1 ml of blood would contain 5×10^7 PRBC.

$$\therefore \text{Dilution factor} = \frac{38.5}{100} \times \frac{4.62 \times 10^9}{5 \times 10^7}$$

$$= 35.57$$

No. of mls of inoculum to be prepared = standardized dose per mouse x No. of mice to be used.

= 0.2 ml x 40

= 8 ml

∴ Amount of blood needed to be taken from donor mouse

$$= \frac{8}{35.57}$$

= 0.2249 ml

∴ 0.225 ml of blood was taken from the donor mouse and diluted to 8 ml with normal saline and 0.2 ml given to each mouse.

3.2.11.6 Drug Administration to Mice

The suspensions of the drugs (aqueous extract in 10% w/v sugar solution) were administered to the mice by the oral route as described below:-

To administer the drugs, the tails and ears of the mice were held with the left hand and then the mice was raised so that the mouth faced the roof. 1 ml syringe containing the drug and tightly attached with sterile rubber tips was gradually but slowly moved in-between the upper and lower set of the teeth of the left side of the head. When satisfied that the drug would go into the stomach, the plunger of the syringe was pressed slowly until the whole drug content went in through the mouth.

3.2.11.7 Test Procedure

Three modes of drug treatment were employed in this study, namely suppressive, curative and prophylactic.

i. Suppressive (4-day Schizonticidal) Treatment Test

In this model, the method of Peters (1980) was employed. 7 groups of infected mice (each group comprised 5 mice) was used. The first group served as control while the groups No. 2 to 4 were administered aqueous extract of tablets produced from the neem bark powders and groups No. 5 to 7 were given extracts obtained from tablets produced from the neem leaves. Three dose levels (50, 200 and 800 mg/kg body weight of the mice) of extracts were employed. The animals were fed through oral route as earlier described 2 hours after inoculation of parasite. On the fifth day, thin blood film from the tail of each mouse was prepared.

ii. Curative (Established infection) Treatment Test

This mode of malaria treatment in experimental animal was carried out according to the approach of Ryley and Peters (1970). Seven groups of infected mice were also used, each group being comprised of 4 mice. All animals were first infected and left for 72 hours (3 days) before commencing of treatment. Thereafter, the extract were administered in three dose levels as previously described. Groups 2, 3 and 4 were administered extracts from the bark tablets while groups 5, 6 and 7 were given leaf tablet extracts. The first group served again as control. The animals were treated for 4 days. Thin blood smears of each animal before treatment were made continuously for 4 days.

iii. Prophylactic Treatment Test

This model was performed as described by Peter (1965). Seven groups of five mice each were utilized. Group 1 was

chosen to serve as the control while groups 2, 3 and 4 were treated with extracts of bark tablets while groups 5, 6 and 7 were given extracts of the leaf tablets. Three dose levels (50, 200 ad 800 mg/Kg body weight of mice) of extracts were also employed as earlier described. They were administered for 3 consecutive days. On the 4th day, all animals were infected and left for 3 days after which thin blood smears were taken from the tails.

3.2.11.8 Preparation of Stained Thin Blood Films

With a clean pair of scissors, a few millimetres of the tail was cut distally from each mouse. The tail was then squeezed until blood drop exuded. The blood was transferred to a clean microscopic slide. The smooth edge of another slide was used at an angle of 45° to spread the blood on the first slide. The smear were fixed with 2 drops of methanol for 30 seconds and left to dry.

The staining solution (Giemsa stain) which was prepared just before staining using pH 7.2 buffer solution (Olise, 1984), was dropped on the slide to cover the blood completely. After 17 minutes, the stain was washed off with the buffer solution and allowed to dry.

3.2.11.9 Evaluation of Parasitaemia

The parasite count of each thin blood film was undertaken under the oil immersion lens of an Optical Microscope (Reichert Scientific Instrument, USA), using a tally counter. In each field, the total number of red blood cells was first counted followed by the counting of parasitized red blood cells. In all the slides, a total of ten

fields were counted and the average percentage of parasitaemia was calculated using the formula given below.

$$\% \text{ Parasitaemia} = \frac{P}{T} \times 100$$

where P = No. of parasitized red blood cells, and

T = Total no. of red blood cells.

3.2.4: Statistical Analysis of Results

Analysis of the results was carried out using the standard error of the mean and any difference in the values was taken to be significant when $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 PHARMACEUTICAL AND PHYSICAL CHARACTERISTICS OF THE BARK AND LEAF POWDERS OF *AZADIRACHTA INDICA* (NEEM TREE)

4.1.1 Particle Size, Size Distribution and Shape of Bark and Leaf Powders

Microscopical examination of the powders showed the leaf powders to be composed primarily of collenchyma tissues which were thickened with longitudinal strips of cellulose. Trichomes and pericyclic fibres were also observed. On the other hand, the bark powders contained fibres, scleroids and cork tissues which were irregularly shaped longitudinal cells that are often thickened with lignin. These compositions of the two powders are consistent with materials obtained from these plant parts (Trease and Evans, 1982).

The particle sizes and size distribution of the neem bark and leaf powders are shown in Appendix A1 and A2 and illustrated graphically in Fig. 4.1. The plot of percentage frequency vs mean particle size showed that both powders are positively skewed with respect to their size distribution. The two curves were almost superimposed on each other, indicating that the bark and leaf powders exhibited similar mean particle sizes and size distribution patterns.

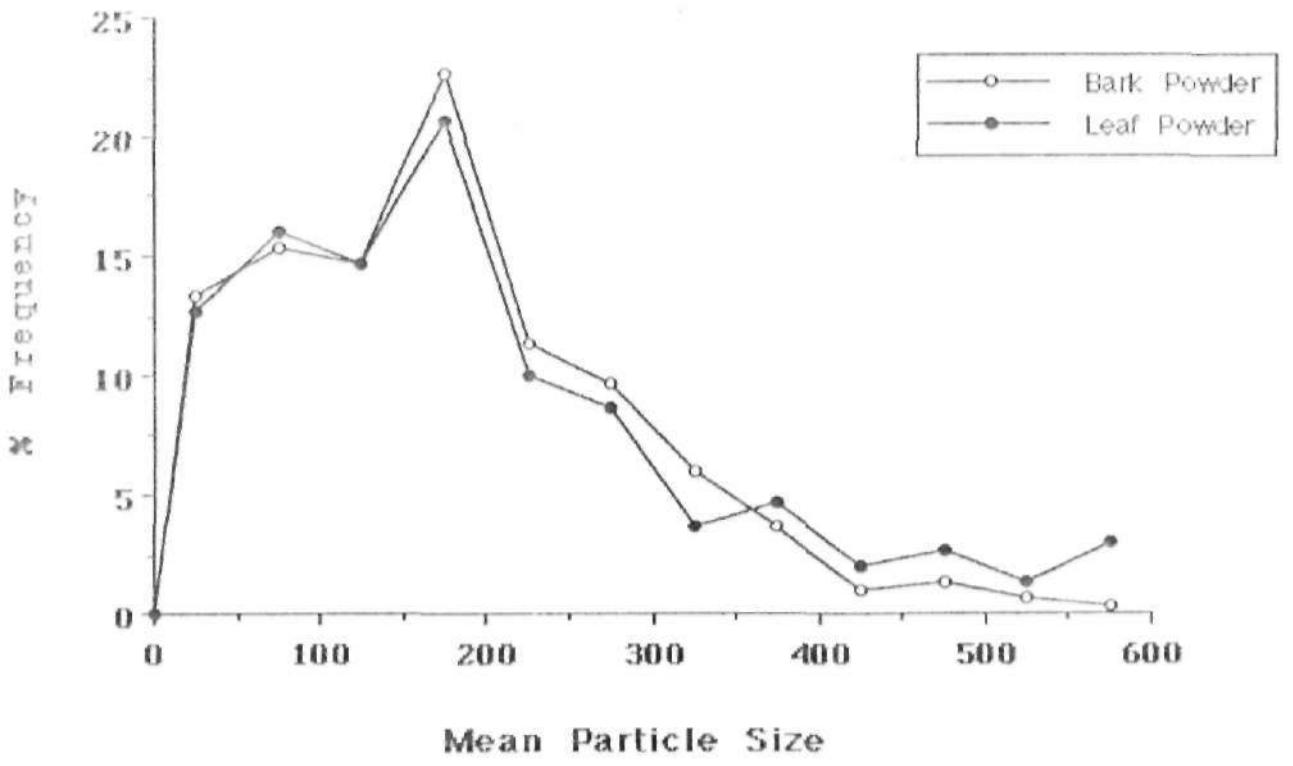


Fig. 4.1: Histogram of Particle Size Distribution Profiles of the Bark and Leaf Powders of Neem Tree.

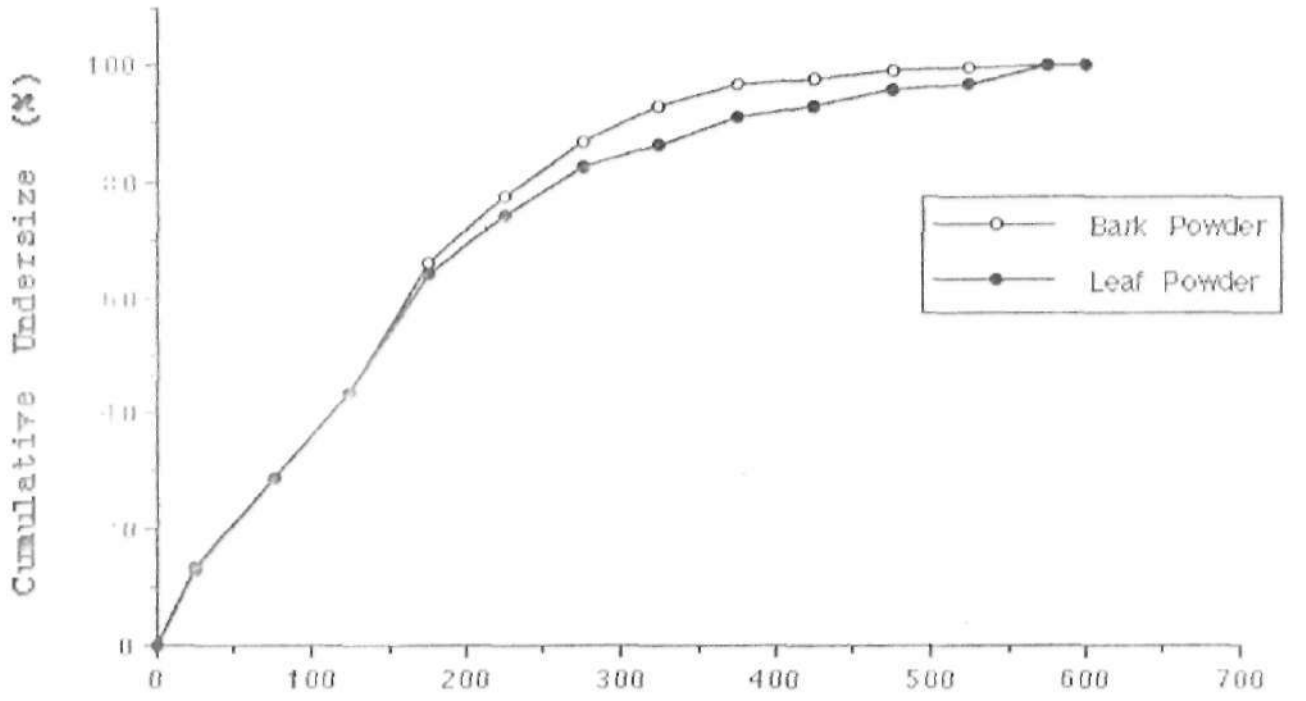


Fig. 4.2: Cumulative Frequency Plot of the Particle Size Distribution of the Bark and Leaf Powders of Azadirachta indica.

Plots of percentage cumulative undersize against mean particle sizes for the bark and leaf powders are depicted in Fig. 4.2. The cumulative undersize plots for the two powders were also very similar. For example, the particle sizes at 50% cumulative undersize for the leaf and bark powders (145 µm and 140 respectively) were relatively very close. Most of the particles have sizes lower than 300 µm. For example, 83% of the leaf and 87% of the bark powders have sizes less than 300 µm.

The apparent similarities in the size and size distribution patterns of the two powders despite the differences in their compositions, may be explained by the fact that both powders were subjected to the same milling processes during the pre-formulation stages. Beside, the major components in the two powders were fibrous materials.

1.2 Moisture Content of the Neem Bark and Leaf Powders

Table 4.1 shows the moisture content values for the two groups of powders. As depicted in this table, both powders had relatively low moisture contents. The moisture content of the bark powders was expectedly higher because of the nature of cork tissues present in bark powders that do not easily release entrapped moisture.

Table 4.1: Moisture Contents of the Neem bark and leaf Powders.

Dry Powder	Moisture Content (%)
Leaf	5.46
Bark	4.36

4.1.3 Flow Properties of the Neem Bark and Leaf Powders

The flow properties of the leaf and bark powders of neem tree determined by the various indices of flow (flow rate, angle of repose and Carrs index) are presented in Tables 4.2, 4.3 and 4.4.

Table 4.2: Flow Rate of the Bark and Leaf Powders of Neem Tree.

Powder	Flow Time (s)	Flow Rate (gm/s)
Leaf	41	1.22
Bark	50	1.00
Binary Mixture (1:1)	53	0.93

Table 4.3: Angles of Repose of the Bark and Leaf Powders of *Azadirachta indica*

Dry Powder	Angle of Repose (°)
Leaf	23.85
Bark	24.54
Binary Mixture (1:1)	24.21

Table 4.4: Carr's Indices of The Bark and Leaf Powders of *Azadirachta indica*

Dry Powder	Carr's Index (%)
Leaf	13.65
Bark	14.39
Binary Mixture (1:1)	14.11

As shown in these tables, values for the bark and leaf powders and their binary mixtures were generally very similar. The flow rate as determined from a flowameter simulates the flow from a hopper to a die-cavity in a tableting machine. The flow rates obtained for the powders were not particularly indicative of very good flow.

The angle of repose, another index of flow, determines to a large extent, the type of inter-particulate forces (internal friction or cohesion of the particles) at play in a powder mass. In most cases, high angle of repose is characteristic of cohesive powders while low value implies non-cohesiveness (Staniforth, 1990). The values obtained in this work indicate that the powders are relatively non-cohesive, i.e they are likely to flow well. Similarly, the Carr's index, another indirect measure of flowability (it is an indicator of the packing geometry of the particles and powder porosity), shows that the bark and leaf powders have fairly good flow. The results of the latter two tests seem to be in contrast with the flow rates. This discrepancy may be due to the irregular shapes of the particles which may interlock to hinder free flow.

The bulk properties of a powder material depend on the particle size distribution. Size, surface area, and surface to volume ratio of a particle are indices of physico-chemical properties of a drug. Generally, as sizes of particles fall below 100 μm, they tend to be cohesive and those with lower than 10 μm hardly flows, while those with sizes greater than 100 μm and up to 250 μm usually possess good flow properties (Staniforth, 1990). Particles with sizes in the range of 100-250 μm constitute 48.67% and 45.32% in the bark and leaf powders respectively. This is most likely to be responsible for the fairly good flow of these powders.

The flowability of a powder is very important in the production of pharmaceutical dosage forms because a free-

flowing powder ensures that there is uniform particle packing and filling of tablet dies which allows tablets to be produced with more consistent physico-chemical properties.

4.2 QUALITY CONTROL ANALYSIS OF TABLETS PRODUCED BY DIRECT COMPRESSION METHOD

Table 4.5 shows the performance of the tablets produced by direct compression from the bark and leaf powders, and their various binary combination on subjection to various quality control parameters

Generally, good quality and pharmaceutically acceptable tablets were obtained when either the bark or the leaf powders were subjected to compression force of 7.5 KN in a tablet press. Tablets produced from the bark powders alone were characterised by high hardness value, non-friability and moderate disintegration time. They disintegrated in less than 9 minutes. In contrast, tablets compressed from the leaf powders were more friable, fairly hard and had shorter disintegration time. Binary mixtures of the two powders in various proportions also gave tablets with properties that were intermediate between those of the bark and leaf.

This unique differences in the properties of the two types of tablets were probably due to the differences in their compositions. The presence of cork tissues which are thickened with lignin may be responsible for the higher mechanical strength for the bark powders.

Incorporation of Avicel^{R} as a diluent in the powders did not improve the quality of the resulting tablets (Table 4.6).

For example, Avicel^{R} could only be incorporated at a maximum proportion of 25% in the leaf powders to produce tablets within reasonably acceptable limits. Tablets produced from the powders containing this content of Avicel^{R} was moderately hard with friability value just above the acceptable 0.8% limit. Tablets produced at higher Avicel^{R} contents were generally very soft, very friable and disintegrated in seconds.

Table 4.5: Properties of Tablets Produced by Direct Compression from Bark and Leaf Powders of *Azadirachta indica*

% of Leaf powder	% of Bark powder	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegration times (min.)
0	100	301.40	3.8	12.0	0.33	8 min. 20 s
25	75	302.00	3.8	7.0	0.32	6 min. 35 s
50	50	299.40	3.8	5.0	0.60	5 min. 34 s
75	25	299.80	3.9	3.0	0.63	5 min. 20 s
100	0	302.00	3.9	3.0	0.65	5 min. 27 s

Pressure setting = 7.5 KN

On the other hand, tablets produced from the bark powders containing Avicel^{R} as diluent have very remarkable good qualities (Table 4.7).

Table 4.6: Properties of Tablets Produced by Direct Compression from Neem Leaf Powders With Avicel^{R} as Diluent.

% of Avicel (as Diluent)	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegration times (min.)
0	299.60	3.9	3.5	0.63	5 min. 17 s
25	299.80	3.9	2.5	0.89	1 min. 40 s
50	300.40	5.2	2.5	3.67	9 s
75	298.40	6.5	0.5	100	6 s
100	299.20	8.3	0.0	100	4 s

Pressure setting = 7.5 KN

Table 4.7: Properties of Tablets produced by Direct Compression Method from Neem Bark Powders with Avicel^{R} as Diluent.

% of Avicel (as Diluent)	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegration times (min.)
0	303.20	3.9	11.5	0.34	8 min. 24 s
25	301.00	3.8	11.0	0.46	6 min. 22 s
50	305.60	3.8	6.0	0.66	4 min. 13 s
75	299.20	5.2	3.5	0.78	30 s
100	299.80	8.2	0.5	57	10 s

Pressure setting = 7.5 KN

Tablets produced from the binary mixtures of the bark and leaf powder (1:1) with Avicel^{R} as the diluent exhibited similar characteristics as those from the leaf powders described in Table 4.6. For example, tablets produced from the binary mixture containing 25% Avicel had friability value greater than the allowed limit.

The type of diluent incorporated was found to have significant influence on the quality of the tablets. This is aptly demonstrated in Tables 4.8 and 4.9, in which Starch R_X 1500 and Lactose were used as diluents respectively. With Starch R_X 1500, good tablets were produced with the leaf powder even at a diluent proportion of 75%. Increasing diluent concentration was found to cause increase in tablet hardness and disintegration times. All tablets produced had friability values within the allowed limit of 0.8%. The reverse trend was the case with tablets produced from the bark powders containing the same diluent (Starch R_X 1500). Tablets could not be produced from the bark powders at very high diluent concentration (75%) at the compression force setting of 7.5 KN.

The detrimental effect of higher concentration of diluent is more pronounced in the case of lactose (Table 4.9). No tablet was producible at lactose proportion of 50% and higher.

Table 4.8: Properties of Tablets Produced by Direct Compression from Bark and Leaf Powders of *Azadirachta indica* with Starch Rx 1500 as Diluent.

% of Starch Rx 1500 (as Diluent)	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegration times (min.)
Leaf Powder:					
0	301.00	3.9	3.5	0.64	5 min. 20 s
25	300.40	3.8	4.0	0.56	8 min. 26 s
50	299.80	3.8	8.0	0.42	12 min. 34 s
75	309.20	3.8	12.0	0.21	20 min. 12 s
100	-	-	-	-	-
Bark Powder:					
0	300.40	3.9	11.5	0.38	8 min. 16 s
25	299.20	3.8	11.0	0.46	7 min. 44 s
50	301.60	3.8	9.0	0.59	6 min. 12 s
75	-	-	-	-	-
100	-	-	-	-	-

(-) indicates that no tablet was formed.
Pressure setting = 7.5 KN

Table 4.9: Properties of Tablets Produced by Direct Compression from Bark and Leaf Powders of *Azadirachta indica* with Lactose as Diluent.

% of Lactose (as Diluent)	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegration times (min.)
Leaf Powder:					
0	300.10	3.9	4.0	0.62	5 min. 28 s
25	299.40	3.8	4.5	0.41	6 min. 36 s
50	-	-	-	-	-
75	-	-	-	-	-
100	-	-	-	-	-
Bark Powder:					
0	299.20	3.8	11.5	0.32	8 min. 26 s
25	300.40	3.9	3.0	1.24	3 min. 12 s
50	-	-	-	-	-
75	-	-	-	-	-
100	-	-	-	-	-

- indicates that no tablet was formed.

Pressure setting = 7.5 KN

The type of diluent and its proportion in the powders generally had significant effect on the qualities of the tablets, particularly on the mechanical strength. It is an established fact that Avicel^{R} is characterised with high compressibility coefficient and poor flowability (Hendel, 1975). It generally yields tablets that are usually very friable with short disintegration times when used in high concentration. This is not unconnected with the high proportion of fines in Avicel^{R} (approximately, half the product passes through a 325 mesh screen) (Hendel, 1975). On incorporation of this diluent into the bark or leaf powder, the proportion of particles with sizes lower than 100 µm will constitute over 50 % of the powder mass. However, in macro-drug formulation as the case in this study, the diluent which is incorporated to improve the flowability and compressibility of the crude powder is normally added in low proportion. It is at this concentration that Avicel was found suitable as diluent in the formulation of tablets of *Azadirachta indica*.

Lactose was generally not found suitable as a filler in the production of tablets from Neem tree. This is due to the intrinsic property of lactose. Lactose is usually characterised by low compressibility and poor flowability. In fact, lactose is more commonly used as a diluent in powders for external application. It is the sprayed-dried lactose that has found wide spread use as a direct compression vehicle.

Starch R_X 1500 when used as diluent, gave the best quality tablets (compared with the Avicel^{R} or lactose) with both the bark and leaf powders of *Azadirachta indica*. The

Granules with smaller size fractions were more easily compressed at lower compression forces than those of larger size fractions. As illustrated in this table, while compression force of 7.5 KN was sufficient to produce tablets from granules of size fraction > 150 < 250 µm, a higher pressure setting of 11.5 KN was required to form tablets from the larger size fraction (> 500 µm).

When methylcellulose was used as the binder, the characteristics of the resulting tablets from the bark powders were remarkably different from those produced with maize starch as binder (Table 4.12). No tablet was formed from the largest size fraction even at a relatively high compression force of 11.5 KN until the binder concentration was raised to 10%. The tablets produced at this concentration were soft, friable, thick and disintegrated within seconds. Tablets with acceptable quality could only be produced from the medium size fraction with this binder. However, when the leaf powder was used, tablets produced from the three size fractions with this binder (methylcellulose) exhibited characteristics desired of good pharmaceutical tablets irrespective of the binder concentration (Appendix B2).

Table 4.10: Properties of Tablets Produced by Wet Granulation from Different Size Fractions of Granules from Leaf Powders of *Azadirachta indica* Prepared with Maize Starch as Binder.

Size Fraction (μm)	Pressure Setting (KH)	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegrat-ion times (min.)
5% Binder paste.						
> 500	8.5	308.00	3.8	4.50	0.34	12 min. 10 s
> 250 < 500	7.0	305.60	3.9	2.5	0.20	10 min. 36 s
> 150 < 250	5.5	298.00	3.9	2.5	0.27	7 min. 34 s
10% Binder paste.						
> 500	8.5	299.60	3.8	5.0	0.26	13 min. 25 s
> 250 < 500	7.0	302.80	3.9	3.0	0.18	12 min. 6 s
> 150 < 250	5.5	300.00	3.9	3.0	0.20	9 min. 22 s
15% Binder paste.						
> 500	8.5	308.80	3.8	5.0	0.16	13 min. 49 s
> 250 < 500	7.0	307.00	3.9	3.0	0.16	12 min. 22 s
> 150 < 250	5.5	304.20	3.9	3.0	0.20	10 min. 12 s
20% Binder paste.						
> 500	8.5	303.20	3.8	5.0	0.16	13 min. 49 s
> 250 < 500	7.0	301.60	3.9	3.0	0.13	12 min. 32 s
> 150 < 250	5.5	299.00	3.9	3.0	0.15	10 min. 26 s

Table 4.11: Properties of Tablets Produced by Wet Granulation from Different Size Fractions of Granules from Bark Powders of *Azadirachta indica* Prepared with Maize Starch as Binder.

Size Fraction (µm)	Pressure Setting (KN)	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegration times (min.)
5% Binder paste						
> 500	11.5	297.20	6.5	0.0	57.21	18 s
> 250 < 500	9.0	301.80	4.3	3.0	0.91	2 min. 5 s
> 150 < 250	7.5	294.80	3.9	6.0	0.31	4 min. 11 s
10% Binder paste						
> 500	11.5	297.80	3.9	3.0	0.51	8 min. 41 s
> 250 < 500	9.0	306.20	4.1	5.0	0.31	4 min. 12 s
> 150 < 250	7.5	292.20	3.8	6.0	0.26	6 min. 42 s
15% Binder paste						
> 500	11.5	297.40	3.9	3.0	0.51	10 min. 24 s
> 250 < 500	9.0	308.00	4.1	5.0	0.28	4 min. 26 s
> 150 < 250	7.5	293.40	3.8	6.0	0.20	6 min. 42 s
20% Binder paste.						
> 500	11.5	298.40	3.9	3.0	0.42	12 min. 59 s
> 250 < 500	9.0	304.60	4.1	5.0	0.21	4 min. 48 s
> 150 < 250	7.5	292.60	3.8	6.0	0.17	8 min. 47 s

Table 4.12: Properties of Tablets Produced by Wet Granulation from Different Size Fractions of Granules from Bark Powder of *Azadirachta indica* Prepared with Methylcellulose as Binder.

Size Fraction (µm)	Pressure Setting (KN)	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegration times (min.)
1.25% Binder mucilage						
> 500	11.5	-	-	-	-	-
> 250 < 500	10.5	297.80	4.2	2.0	0.94	11 s
> 150 < 250	8.0	295.20	4.0	0.0	59.20	23 s
2.5% Binder mucilage						
> 500	11.5	-	-	-	-	-
> 250 < 500	10.5	298.80	4.2	3.0	0.88	2 min. 19 s
> 150 < 250	8.0	299.60	4.0	0.5	41.6	36 s
5.0% Binder mucilage						
> 500	11.5	-	-	-	-	-
> 250 < 500	10.5	300.20	4.0	5.5	0.20	4 min. 12 s
> 150 < 250	8.0	298.80	3.8	1.0	12.7	39 s
10% Binder mucilage.						
> 500	11.5	300.60	5.8	0.5	63.24	10 s
> 250 < 500	10.5	300.40	4.0	5.5	0.20	4 min. 40 s
> 150 < 250	8.0	299.90	3.8	1.0	6.8	50 s

Table 4.13: Properties of Tablets Produced by Wet Granulation from Different Size Fractions of Granules from Leaf Powders of *Azadirachta indica* Prepared with Polyvinyl pyrrolidone as Binder.

Size Fraction (μm)	Pressure Setting (KN)	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegration times (min.)
5% Binder solution						
> 500	9.0	297.60	3.8	3.5	0.20	10 min. 16 *s*
> 250 < 500	7.0	290.60	3.8	3.0	0.21	8 min. 28 *s*
> 150 < 250	6.0	299.00	3.9	3.0	0.28	6 min. 14 *s*
10% Binder solution						
> 500	9.0	293.20	3.8	4.0	0.21	10 min. 28 *s*
> 250 < 500	7.0	296.60	3.8	3.5	0.20	9 min. 10 *s*
> 150 < 250	6.0	296.80	3.9	3.0	0.20	7 min. 28 *s*
15% Binder solution						
> 500	9.0	303.40	3.8	4.0	0.17	11 min. 32 *s*
> 250 < 500	7.0	295.00	3.8	3.5	0.17	10 min. 26 *s*
> 150 < 250	6.0	299.60	3.9	3.0	0.18	8 min. 11 *s*
20% Binder solution.						
> 500	9.0	297.40	3.8	4.0	0.15	12 min. 42 *s*
> 250 < 500	7.0	296.00	3.8	3.5	0.17	11 min. 36 *s*
> 150 < 250	6.0	296.00	3.9	3.0	0.17	9 min. 22 *s*

Good quality tablets were also produced with polyvinyl pyrrolidne binder from the leaf powders (Table 4.13). The tablet hardness, friability values and thickness for the three size fractions were virtually the same at each binder concentration, though different compression forces were employed to form tablets from the size fractions. Tablets produced from the bark powders with this binder showed characteristics that were in many respects similar to those of the leaf powder (Appendix B3). The resulting tablets had however, higher hardness values and those tablets produced using 5% binder exhibited higher friability values, which were in most cases over the allowed limit of 0.8%.

As these results showed, increasing concentration of binder solution generally leads to increasing tablet hardness and decreasing friability values. As observed by Kunle (1988) with maize starch paste, the increase in binding capacity due to increasing binder concentration is only applicable up to a certain concentration. The decreased friability due to increasing binder concentration is attributed to increased degree and strength of inter-particulate bonding (Esezobo and Ambujam, 1982, Iwuagwu *et al*, 1986). The observed increase in disintegration times with increasing binder concentration which is consistent with previous works is ascribed to increasing binding force of the granule in the tablet compact and may also be due to the formation of a thin mucilaginous film that may constitute a viscous barrier between the tablets or its break-up fragments and water (Esezobo, 1986).

In all the three binders, there were marked differences in the performance of the tablets produced from the bark and the leaf powders. This might invariably be due to the constituents of the two powders as had earlier been explained (4.1, page 61). The bark powders is made up of irregularly shaped, hard fibrous cork tissues, lignin and cellulose materials. This might mostly be responsible for the higher compression force required to form tablets from the bark powders as compared with leaf powders, which contained less proportion of fibrous tissues. The differences in the tablets produced by the three binders could also be ascribed to the nature of the binders. For example, while maize starch and polyvinyl pyrrolidone binders generally gave good quality tablets, tablets could not be formed from bark powders with methylcellulose mucilage. The inability of methylcellulose mucilage to form tablets from the bark powders might be attributed to some similarity in the constituent of this binder and the bark powder. The proportion of irregularly shaped and longitudinal cellulose fibres will be increased in the bark granules when methylcellulose (a fibrous, irregularly shaped particle) was used as the binder. Invariably, the inter-particulate friction which opposes the binding of the particles together is enhanced, hence compacts could not be made.

As indicated in the results, higher compression force was required to form tablets from the larger particle size fractions. This might be because during the compression process, some of the compression forces are dissipated in breaking down the larger particles into smaller units before powder consolidation could take place. As such, the larger

the particle, the higher compression force required to form tablet. The importance of particle size fraction in tablet technology has long been realised. Barber (1993) reported that the tableting processes depend among other things on the size distribution of the material involved.

4.4 MICROBIOLOGICAL STUDIES ON THE BARK, LEAF AND TABLETS OF *AZADIRACHTA INDICA*

The bacterial contamination levels and flora of the leaf and bark powders of *Azadirachta indica* as shown in Table 4.14 indicates that they are moderately contaminated with spore-forming and non-sporing bacillus organisms. Macro- and Micro-scopical examination also showed that the predominant fungal organism in the bark powders was *Mucor* while *Aspergillus* sp. was isolated from the leaf powder. The microbial contamination levels of the tablets produced by direct compression are illustrated in Table 4.15. As depicted in this table, the fungal counts were much higher than those obtained with the initial crude powders of the plant. On the contrary, the bacterial counts of the tablets produced using lactose as diluent was also much lower than the values obtained for the crude drugs, while the counts for the tablets produced using other diluents were not much different. The major bacterial contaminants were again Bacillus sp. while *Aspergillus* and *Mucor* were the predominant fungal contaminants. Tablets produced by the wet granulation method were characterised by low bacterial and high fungal counts (Table 4.16), with *Bacillus* sp. and

Aspergillus with *Mucor* as the major bacterial and fungal organisms respectively.

Table 4.14: Microbial Contamination Levels and Flora of the Bark and Leaf Powders of *Azadirachta indica*.

Powder	Bacterial organisms		Fungal Organisms	
	Count (cfu/g)	Macro- and Micro-scopical Character-istics	Count (cfu/gm)	Macro- and Micro-scopical Character-istics
Bark Powder	438 ± 1.003	Gram positive spore-forming rods in chains, with cream-coloured lobed colony. May be *Bacillus*	117 ± 1.404	White fluffy colony with septate mycellium. *Aspergillus*
Leaf powder	308 ± 2.041	Single occurring gram positive rods; Colonies are with cream and circular. Likely *Bacillus*	50	Off-white colonies exhibiting pseudo-mycelium. May be Yeast.

Results of test for indicator organisms showed that *E. coli*, *Staph. aureus*, *Ps. aeruginosa* and *Salmonella* sp. were not present in the various test samples (bark, leaf and their various tablets)

Studies on the antimicrobial property of this plant showed that the plant exerts some moderate inhibitory activities against wide range of micro-organisms (Table 4.17). The ethanolic extracts of the neem (the bark and leaf powders) were generally more potent than the aqueous extracts, the former being active against the five test micro-organisms, namely *B. subtilis*, *E. coli*, *Ps. aeruginosa*, *Staph. aureus* and *C. albicans*. The greatest inhibitory activities was exerted against *B. subtilis* and *E. coli* followed by *C. albicans*. Even high concentration of the aqueous extract (10% w/v) either did not show any inhibition or only exerted feeble antimicrobial activity against the other test organisms. *Ps. aeruginosa* was the least susceptible to inhibitory action of the various extracts. The results also indicated that the extracts from the bark powder was more potent in the degree of antimicrobial activity than those from the leaf powder.

The predominance of *Bacillus* sp. and the fungal organisms- *Aspergillus* and moulds in the bark and leaf powders is not unexpected. This is because, the powders during processing (drying, size-reduction, mixing, granulation, tableting, etc) were constantly exposed to the atmosphere which is laden with these organisms. This is especially so in the dry humid and windy environment that characterise the research area. The higher levels of *Bacillus* organisms in the bark powders may be ascribed to the closeness of this plant part to the soil- a natural habitat for this organism.

Table 4.15: Microbial Contamination Levels and Flora of Tablets Produced by Direct Compression Method

Powder and Diluent Type	Bacterial organisms		Fungal Organisms	
	Count (cfu/g)	Macro- and Micro-scopical Character-istics	Count (cfu/gm)	Macro- and Micro-scopical Character-istics
Bark Powder (75%) + 25% Starch Rx 1500	410 ± 1.031	Gram positive rod, in chains, with cream-coloured lobed colony. May be *Bacillus*	310	White fluffy colony with coeno-cytic mycelium. Likely Mucor.
Bark powder (75%) + Lactose (25%)	95 ± 2.044	Gram positive spore-forming rods in chains; with cream circular colonies. Likely *Bacillus*	520	White fluffy colony with septate mycelium. May be *Aspergillus*
Leaf powder (75%) + Lactose (25%)	75 ± 3.021	Single gram positive rods; with cream circular colonies. May be *Bacillus*	480	white fluffy growth, with septate mycelium. Likely *Aspergillus*
75% Bark powder + 25% Avicel	280 ± 0.962	Gram positive spore-forming rods in chains. Produce cream, lobed colonies. May be *Bacillus*	250	white fluffy growth with non-septate mycelium. May be Mucor.
75% Leaf powder + 25% Avicel	0	-	120	white fluffy growth on plate with septate mycelium. May be *Aspergillus*

Table 4.16: Microbial Contamination Levels and Flora of Tablets Produced by Wet Granulation Method

Powder and Binder Type	*Bacterial organisms*		*Fungal Organisms*	
	Count (cfu/g)	Macro- and Micro-scopical Character-istics	Count (cfu/gm)	Macro- and Micro-scopical Character-istics
Leaf Powder + 10% Maize starch	20	Gram positive rods in singles, with cream-coloured circular colony. May be *Bacillus*	0	
Bark powder + 10% Maize Starch	10	Gram positive spore-forming rods in chains; with cream circular colonies. May be *Bacillus*	270 ± 2.041	White colony with pseudomycelium. May be yeast.
Bark powder + 2.5% methylcellulose	0	-	230 ± 1.003	white fluffy growth, covering the plate, with septate mycelium. May be *Aspergillus*.
Leaf powder + 2.5% methylcellulose	0	-	235 ± 3.021	white fluffy growth with septate mycelium. May be *Aspergillus*
Bark powder + 10% Polyvinyl pyrrolidone	120	Gram positive spore-forming rods, in chains, with cream and lobed colonies. Likely *Bacillus*	370 ± 1.036	white fluffy colony with coenocytic mycelium. May be Mucor.

Table 4.17: Mean Diameters of Zones of Inhibition of Bark and Leaf Powders of *Azadirachta indica*

Test Micro-organism	Diameter of Zones of Inhibition (mm)			
	Aqueous Extract		Ethanol Extract	
	1% w/w	10% w/w	1% w/w	10% w/w
Bark Powder				
B. subtilis	11.50	14.50	18.50	21.00
E. coli	0.00	17.00	16.50	21.50
Ps. aeruginosa	0.00	12.00	14.00	15.50
Staph. aureus	0.00	13.00	15.50	17.50
C. albicans	12.00	16.50	15.00	19.00
Leaf Powder				
B. subtilis	0.00	11.50	14.50	17.00
E. coli	0.00	10.50	13.50	16.50
Ps. aeruginosa	0.00	0.00	11.50	14.50
Staph. aureus	0.00	11.0	14.00	16.00
C. albicans	0.00	0.00	13.00	16.50

The observed antimicrobial activities of the extracts confirms the reports of Schneider (1986) who indicated that Azadirachta indica possesses pronounced activity against *Staphylococcus aureus*, *Staph. coagulase*, *B. subtilis* and diphtheroids. The antimicrobial activity was attributed to the presence of nimbolide and nimbic acid in the extracts.

4.5 PHARMACOLOGICAL SCREENING OF TABLETS OF *AZADIRACHTA INDICA* FOR ANTIMALARIAL ACTIVITY

Results of the pharmacological screening for anti-malarial activity of the tablets produced from *Azadirachta indica* are shown in Tables 4.18 to 4.20 and in Fig. 4.3 to 4.6.

Table 4.18: The 4-day Schizonticidal (Suppressive) Effect of Aqueous Extracts of *Azadirachta indica* (Leaf and Bark) on *Plasmodium yoelii nigeriensis*

Dose Level (mg/Kg body weight)	Parasitaemia (% ± SEM)	Average % Reduction in Parasitaemia	Level of Significance
LEAF			
0 (control)	50.220 ± 0.404	-	-
50	24.750 ± 1.047	50.72	S
200	20.580 ± 0.244	59.02	S
800	10.200 ± 0.200	79.69	S
BARK			
0 (control)	50.220 ± 0.404	-	-
50	32.300 ± 1.537	35.68	S
200	29.300 ± 0.579	51.66	S
800	15.960 ± 0.242	68.22	S

$p < 0.05$

SEM = standard error of the mean

S = significant

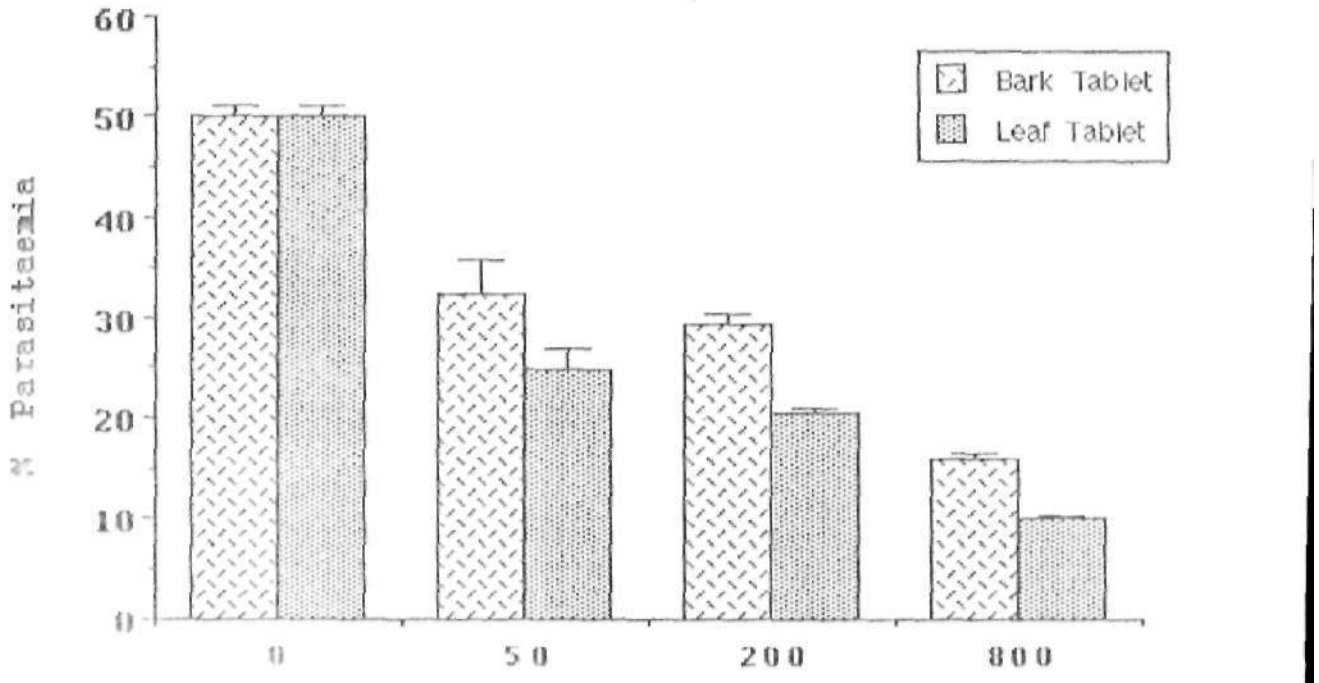


Fig 4.3: Antimalarial Suppressive Effect of Aqueous Extracts of the Bark and Leaves of Neem Tree on Established Malaria Infection of Plasmodium yoelii nigeriensis in Mice.

Table 4.19: The Prophylactic Effect of Aqueous Extracts of *Azadirachta indica* (Leaf and Bark) on *Plasmodium yoelii nigeriensis*

Dose Level (mg/Kg body weight)	Parasitaemia (% ± SEM)	Average % Reduction in Parasitaemia	Level of Significance
LEAF			
0 (control)	55.780 ± 3.630	-	-
50	23.140 ± 0.181	58.52	S
200	14.920 ± 0.414	73.25	S
800	13.740 ± 0.641	75.37	S
BARK			
0 (control)	55.780 ± 3.630	-	-
50	26.920 ± 0.292	51.74	S
200	22.400 ± 0.217	59.84	S
800	19.180 ± 0.427	65.62	S

p < 0.05

SEM = standard error of the mean

S = significant

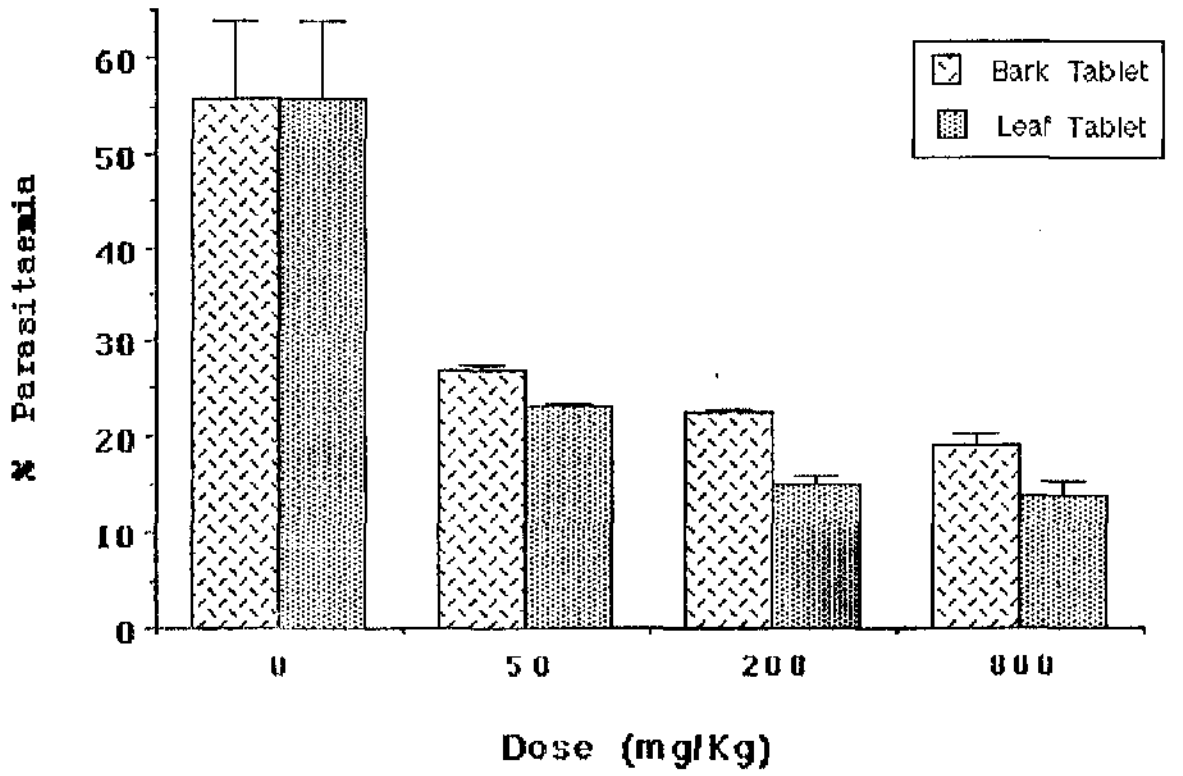


Fig. 1.4: Antimalarial Prophylactic Effect of Aqueous Extracts of the Bark and Leaves of Neem Tree on Established Malaria Infection of Plasmodium yoelii nigeriensis in Mice.

As depicted in Table 4.18 and Fig. 4.3, *Azadirachta indica* possess appreciable antimalarial suppressive effect. At an extract dosage of 50 mg/Kg body weight, parasitaemia was reduced by 35.68% and 50% in tablets produced from the bark and leaf tablets respectively. Higher concentrations of the drug extract significantly elicited greater degree of suppression. Generally, the leaf extracts were more effective as schizonticidal agent.

Data on the screening for the prophylactic effect of the plant in the treatment of malaria as shown in Table 4.19 and Fig. 4.4, also demonstrates that the leaf extract caused greater reduction in parasitaemia than the bark extract at the three test concentrations. For example, while average reduction in parasitaemia was 73.25% for the leaf extract at drug concentration of 200 mg/Kg body weight, the percentage reduction in parasitaemia for the bark extract was 59.84%. At the three dosage levels, the percentage reduction in parasitaemia were significant.

Table 4.20 and Figs. 4.5 and 4.6 show the results of the pharmacological screening of the plant (*Azadirachta indica*) in the application of curative treatment of malaria. As indicated in this table, the mice generally die once the percentage parasitaemia has risen up to about 80%. While the untreated infected mice died within 48 hours (2 days), those treated with dosage level of 50 mg/Kg body weight survived for 72 hours (3 days). At much higher dosage level of the extract (800 mg/Kg), the life span of the infected mice was extended further by a day (i.e. survived for 4 days).

Table 4.20: Percentage Parasitaemia of *Plasmodium yoelii nigeriensis*-infected Mice Subjected to Curative Treatment with Aqueous Extracts of *Azadirachta indica*

Dose Level (mg/Kg)	Plant Part	Post Infection/Initial Treatment Days				
		3 (0)*	4 (1)*	5 (2)*	6 (3)*	7 (4)*
0 (control)	-	44.90 ± 0.216	54.80 ± 0.580	80.75 ± 0.119	-	-
50	Leaf	35.175 ± 0.328	43.150 ± 0.048	65.05 ± 0.132	70.250 ± 0.553	-
	Bark	41.550 ± 0.744	45.325 ± 0.048	65.050 ± 0.132	77.200 ± 2.400	-
200	Leaf	29.175 ± 0.229	40.550 ± 0.202	50.425 ± 0.269	68.550 ± 0.250	-
	Bark	34.625 ± 0.202	40.375 ± 0.225	57.325 ± 0.202	76.067 ± 0.677	-
800	Leaf	25.050 ± 0.247	38.250 ± 0.284	49.925 ± 0.193	64.800	81.150 ± 1.050
	Bark	34.025 ± 0.898	38.275 ± 0.317	54.300 ± 0.280	66.633 ± 1.192	84.400

* Values in parenthesis () represent days after initial treatment.

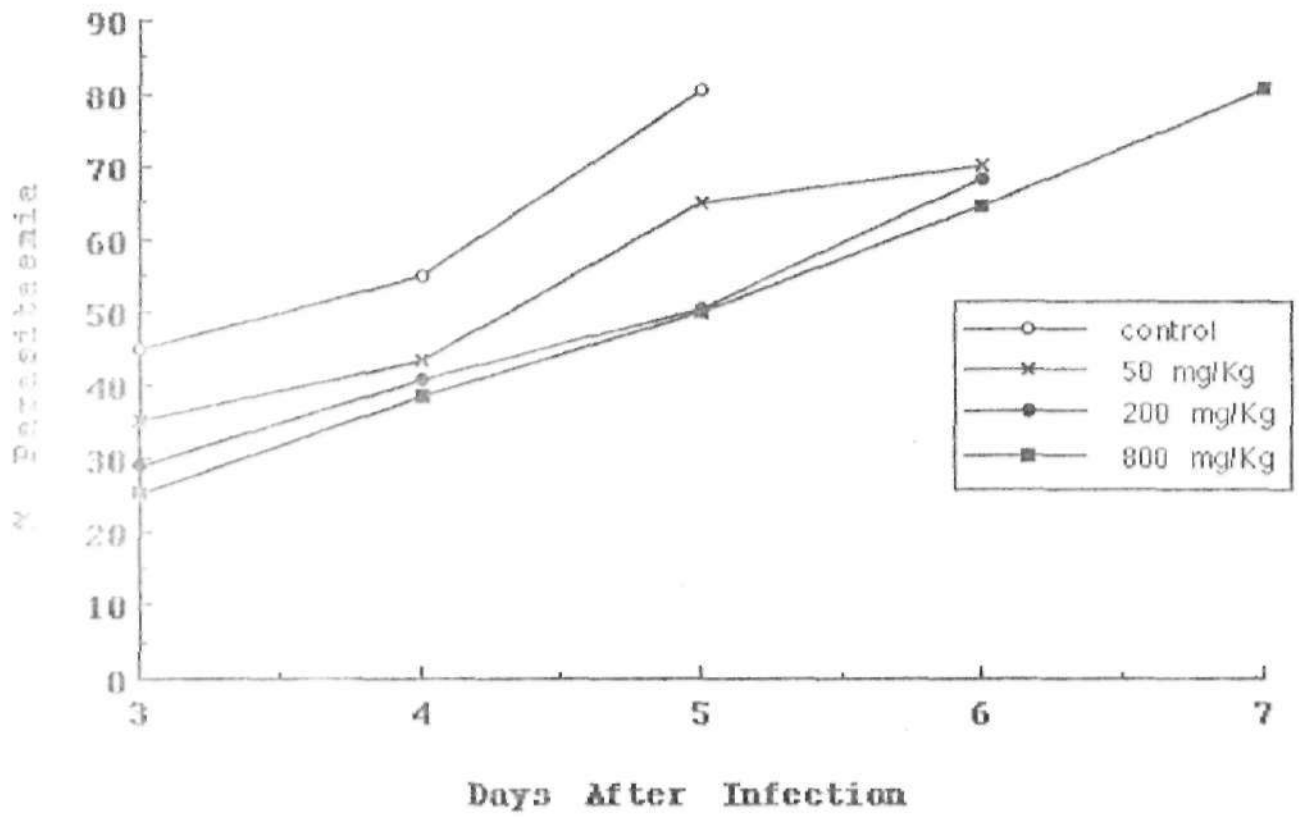


Fig. 4.5: Antimalarial Curative Effect of Aqueous Extracts of the Leaves of Neem Tree on Established Malaria Infection of Plasmodium yoelii nigeriensis in Mice.

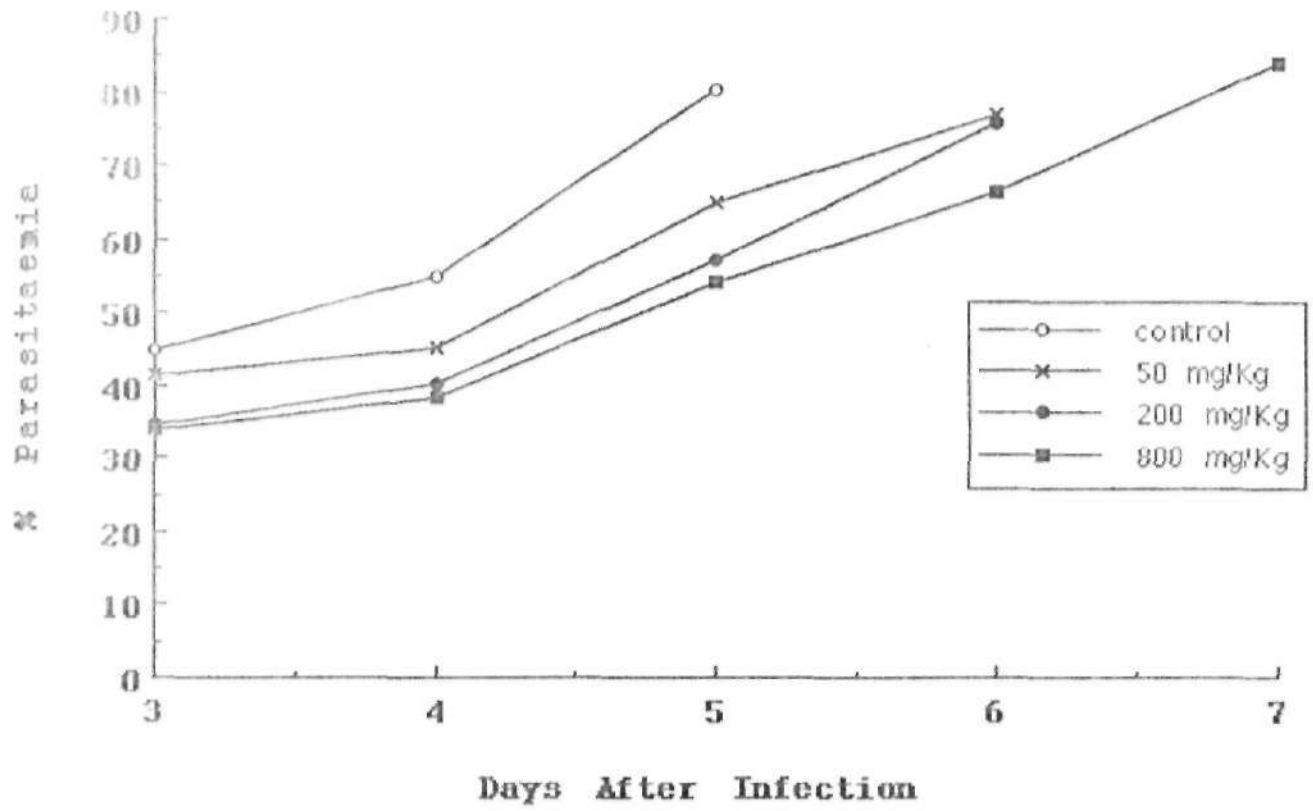


Fig. 4.6: Antimalarial Curative Effect of Aqueous Extracts of the Barks of Neem Tree on Established Malaria Infection of Plasmodium yoelii nigeriensis in Mice.

As revealed by the pharmacological screening, *Azadirachta indica* exerts pronounced prophylactic and suppressive antimalarial activity, but has no curative effect. Iwalewa *et al* (1992) also reported similar findings. The antimalarial activity of this plant has been attributed to the presence of β-sitosterol (Basak and Chakraborty, 1968); nimbolide (Ekong *et al*, 1971); gedunin and other limonoids (Khalid *et al*, 1989).

Extrapolation of the data obtained from the pharmacological screening of the plant in plasmodium yoelii nigeriensis-infected mice, revealed that an adult human being needs to ingest minimally a total of 155 tablets per day (at 200 mg/Kg body weight) each of 300 mg weight before a significant antimalarial effect is exerted. For a more pronounced antimalarial activity, 621 tablets (equivalent to 800 mg/Kg body weight) needs to be consumed on a daily basis. This amounts to 48 gm of the powdered material, which seems to be in disagreement with the common practice by the populace that uses a handful of the leaves to prepare concoctions to be used for several days. This lack of correlation between the dose obtained in this study by extrapolation from the pharmacological screening and the common practice may be due to several factors, namely:-

- the use of a wrong parasite. In humans, the causative agents of malaria are: *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium vivax*. These malaria parasites do not cause infection in rodents. Similarly, *Plasmodium yoelii nigeriensis*, which is used in this study causes malaria only in rodents but not in

humans, hence extrapolating activity in such a rodent parasite to human malaria parasite may not be realistic. The use of the rodent parasite, *Plasmodium yoelii nigeriensis*, in modelling the responses to human malaria infection is predicated on the fact that this parasite is sensitive to chloroquine, the standard drug used in the screening for antimalarial activity. The constituents of *Azadirachta indica* that have so far been suggested to be responsible for the antimalarial activity has a different chemical structure from chloroquine, and are not likely to have similar modes or mechanism of antimalarial action.

- the use of a wrong test animal. The patho-physiology of and the biochemical systems in mice and humans are very different and therefore, a plant constituent exhibiting pronounced pharmacological activity in one animal model may be ineffective in another animal with different pathological and anatomical system.

CHAPTER FIVE

CONCLUSION

The pharmaceutical properties of the bark and leaf powders such as particle size and size distribution, flowability and moisture contents were in most cases similar. The differences in their composition was found to greatly affect the properties of their compacts particularly when tablet adjuvants were incorporated. Both direct compression and wet granulation methods were found to give pharmaceutically acceptable tablets. Although bark powders alone could be compressed to give very hard tablets, better quality tablets were produced when Avicel^{R} (micro-crystalline cellulose) was used as a diluent in the direct compression process. Lactose and Starch R_{X} 1500 were not suitable as diluents in the production of tablets from *Azadirachta indica*.

Tablets produced by the wet granulation method were generally of better quality. Tablets could be produced from the bark and leaf powders of *Azadirachta indica* when maize starch or polyvinyl pyrrolidone is used as a binder at a minimal 10% w/w concentration. Methylcellulose was not able to give good tablets when used as a binder in the production of tablets from bark powders. The granule size fraction was also found to play some roles in the production of tablets from powdered leaves and barks of neem trees. Relatively large size granules required higher compression pressures to form compacts and most of the tablets produced were friable

with very short disintegration times. Granules of > 250 µm but < 500 µm size range gave the best tablets.

The powders (bark and leaf) were moderately contaminated with *Bacillus* spp, *Aspergillus* and moulds. No indicator or pathogenic micro-organisms was found in the powders. Exposure to the atmosphere during processing greatly increased the level of contamination especially of the fungal organisms. The aqueous and ethanol extracts of the bark and leaf parts of the plant had significant inhibitory effect mostly on gram positive organisms and yeast cells with little or no activity on *Ps. aeruginosa*.

Results of the pharmacological tests showed that *Azadirachta indica* possesses appreciable prophylactic and suppressive antimalarial effects but lacks curative property in the management of malaria infection. The leaf powder was more effective than the bark powder in their antimalarial properties. Calculation of dosage regimen indicates that an adult human being will need to ingest minimally, 48 g of the powder per day, an amount that is impracticable. In view of this, further formulation studies may need to be carried out using the extracts of the plant instead of the crude drug powder. It will also be desirable if the antimalarial activity screening can be performed on human volunteers instead of animal models, so that a more realistic data for use in the determination of dosage regimen can be gathered.

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APPENDICES

Appendix A1

Particle Size Distribution of Powdered Neem Leaf

Size Range (µm)	Mean Size Range (µm)	Frequency (n)	Percentage Frequency	Cumulative % Undersize
0 - 50	25	38	12.67	12.67
51 - 100	75	48	16.00	28.67
101 - 150	125	44	14.67	43.34
151 - 200	175	62	20.67	64.01
201 - 250	225	30	10.00	74.01
251 - 300	275	26	8.67	82.68
301 - 350	325	11	3.66	86.34
351 - 400	375	14	4.67	91.01
401 - 450	425	6	2.00	93.01
451 - 500	475	8	2.66	95.67
501 - 550	525	4	1.33	97.00
551 - 600	575	9	3.00	100.00

$\Sigma n = 300$

Appendix A2

Particle Size Distribution of Powdered Neem Bark

Size Range (µm)	Mean Size Range (µm)	Frequency (n)	Percentage Frequency	Cumulative % Undersize
0 - 50	25	40	13.33	13.33
51 - 100	75	46	15.33	28.66
101 - 150	125	44	14.67	43.33
151 - 200	175	68	22.67	66.00
201 - 250	225	34	11.33	77.33
251 - 300	275	29	9.67	87.00
301 - 350	325	18	6.00	93.00
351 - 400	375	11	3.67	96.67
401 - 450	425	3	1.00	97.67
451 - 500	475	4	1.33	99.00
501 - 550	525	2	0.67	99.67
551 - 600	575	1	0.33	100.00

$\Sigma n = 300$

Appendix B1

Properties of Tablets Produced by Wet Granulation from Different Size Fractions of Granules from Binary Mixtures of Bark and Leaf Powders Prepared with Maize Starch as Binder.

Size Fraction (μm)	Pressure Setting (KN)	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegrat-ion times (min.)
5% Binder paste						
> 500	11.5	292.20	5.2	0.5	2.94	40 s
> 250 < 500	9.0	294.60	4.2	5.5	0.30	4 min. 16 s
> 150 < 250	7.0	295.40	3.9	5.0	0.29	5 min. 28 s
10% Binder paste						
> 500	11.5	293.60	4.0	5.5	0.29	10 min. 22 s
> 250 < 500	9.0	299.00	3.8	6.0	0.25	7 min. 18 s
> 150 < 250	7.0	295.80	3.8	5.0	0.27	5 min. 36 s
15% Binder paste						
> 500	11.5	295.80	3.8	6.0	0.20	12 min. 26 s
> 250 < 500	9.0	299.40	3.8	6.0	0.18	11 min. 28 s
> 150 < 250	7.0	297.00	3.8	5.0	0.20	10 min. 44 s
20% Binder paste.						
> 500	11.5	296.40	3.8	6.0	0.19	12 min. 47 s
> 250 < 500	9.0	301.60	3.8	6.0	0.13	11 min. 58 s
> 150 < 250	8.0	298.40	3.8	5.0	0.17	11 min. 14 s

Appendix B2

Properties of Tablets Produced by Wet Granulation from Different Size Fractions of Granules from Leaf Powders Prepared with Methylcellulose as Binder.

Size Fraction (μm)	Pressure Setting (KN)	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegrat -ion times (min.)
1.25% Binder mucilage						
> 500	9.0	300.00	3.9	4.0	0.20	10 min. 45 s
> 250 < 500	8.0	293.60	3.8	4.0	0.27	7 min. 28 s
> 150 < 250	6.0	294.00	3.8	3.0	0.20	9 min. 35 s
2.5% Binder mucilage						
> 500	9.0	302.80	3.9	4.5	0.17	12 min. 15 s
> 250 < 500	8.0	295.60	3.8	4.0	0.25	7 min. 50 s
> 150 < 250	6.0	295.60	3.8	3.0	0.20	10 min. 7 s
5.0% Binder mucilage						
> 500	9.0	306.40	3.8	5.0	0.16	12 min. 21 s
> 250 < 500	8.0	295.20	3.8	4.0	0.20	7 min. 55 s
> 150 < 250	6.0	298.40	3.8	3.0	0.17	10 min. 26 s
10% Binder mucilage.						
> 500	9.0	301.60	3.8	5.0	0.09	14 min. 32 s
> 250 < 500	8.0	299.60	3.8	4.0	0.17	8 min. 10 s
> 150 < 250	6.0	304.20	3.8	3.0	0.13	12 min 5 s

Appendix B3

Properties of Tablets Produced by Wet Granulation from Different Size Fractions of Granules from Bark Powders Prepared with Polyvinyl pyrrolidone as Binder.

Size Fraction (μm)	Pressure Setting (KN)	Mean Weight (mg)	Thickness (mm)	Hardness (Kgf)	Friability (%)	Disintegration times (min.)
5% Binder mucilage						
> 500	11.0	300.00	5.5	0.5	56.67	5 s
> 250 < 500	9.0	295.20	4.0	4.0	0.95	3 min. 12 s
> 150 < 250	7.0	297.80	4.0	4.0	0.79	4 min. 26 s
10% Binder mucilage						
> 500	11.0	309.60	4.3	6.0	0.22	8 min. 36 s
> 250 < 500	9.0	299.60	4.0	4.0	0.27	6 min. 5 s
> 150 < 250	7.0	297.80	3.9	4.5	0.17	8 min. 24 s
15% Binder mucilage						
> 500	11.0	300.80	4.3	6.0	0.22	10 min. 10 s
> 250 < 500	9.0	299.20	4.0	4.0	0.23	8 min. 10 s
> 150 < 250	7.0	297.20	3.9	4.5	0.17	8 min. 44 s
20% Binder mucilage.						
> 500	11.0	303.80	4.3	6.0	0.19	14 min. 12 s
> 250 < 500	9.0	299.80	4.0	4.0	0.17	10 min. 9 s
> 150 < 250	7.0	297.40	3.9	4.5	0.13	12 min. 46 s

Appendix C1

Weights of Test Mice and Average Quantity of Drug Solution Used in the Antimalarial Screening

Cage Number	Weight of each Mouse in the Cages (gm)					Average Weight per Cage (gm)	Average Quantity of Drug administered (ml)
	M1	M2	M3	M4	M5		
Suppressive Treatment							
1	22	24	24	24	24	23	-
2	20	23	24	24	24	23	0.029
3	19	22	20	18	18	19.4	0.097
4	24	24	20	24	23	23	0.460
5	19	18	23	22	21	20.6	0.026
6	23	24	18	18	24	21.4	0.107
7	18	24	24	19	18	20.6	0.412
Prophylactic Treatment							
1	24	20	24	18	20	21.1	-
2	18	24	21	24	18	21.0	0.026
3	24	24	20	23	18	21.8	0.109
4	21	24	23	21	18	21.4	0.428
5	24	20	24	22	18	21.6	0.027
6	22	24	21	24	18	21.8	0.109
7	21	24	18	18	20	20.2	0.404
Curative Treatment							
1	23	20	18	24		21.25	-
2	18	18	20	24		20.00	0.025
3	18	24	24	24		22.50	0.113
4	18	24	23	24		22.25	0.445
5	24	22	18	24		22.00	0.028
6	24	23	23	19		22.25	0.111
7	18	24	18	20		20.00	0.400

Appendix C2

Percentage Parasitaemia in Mice Subjected to Suppressive and Prophylactic Antimalaria Treatment with Extracts of the Leaf and Bark of *Azadirachta indica*.

Type of Treatment	Dose Level (mg/Kg)	Parasitaemia (%) M1	M2	M3	M4	M5	Mean ± SEM
Suppressive Treatment	**Leaf**						
	0 (control)	49.9	51.5	50.8	49.5	49.4	50.22 ± 0.404
	50	26.3	25.9	25.1	1.7	-	24.75 ± 1.047
	200	20.3	20.4	20.1	21.5	20.6	20.58 ± 0.244
	800	10.4	10.0	-	-	-	10.20 ± 0.200
	Bark						
	0 (control)	49.9	51.5	50.8	49.5	49.4	50.22 ± 0.404
	50	37.2	30.7	34.3	30.8	28.5	32.30 ± 1.537
	200	28.8	28.2	30.9	29.3	-	29.30 ± 0.579
	800	16.5	16.6	15.6	15.5	15.6	15.96 ± 0.242
Prophylactic Treatment	**Leaf**						
	0 (control)	48.2	49.1	66.9	53.3	61.4	55.78 ± 3.630
	50	23.4	23.5	23.0	22.5	23.3	23.14 ± 0.161
	200	14.8	14.6	15.9	13.6	15.7	14.92 ± 0.414
	800	13.1	15.7	12.0	13.3	14.6	13.74 ± 0.641
	Bark						
	0 (control)	48.2	49.1	66.9	53.3	61.4	55.78 ± 3.630
	50	26.2	26.6	26.7	27.2	27.9	26.92 ± 0.292
	200	23.1	22.6	22.4	21.9	22.0	22.40 ± 0.217
	800	19.9	17.7	20.1	18.9	19.3	19.18 ± 0.427

Appendix C3

Parasitaemia (%) in Mice Subjected to Curative (Established Infection) Antimalaria Treatment with Extracts of the Leaf and Bark of *Azadirachta indica*.

Dose Level (mg/Kg)	Plant Part	Post Infection/Initial Treatment Days				
		3 (0)*	4 (1)*	5 (2)*	6 (3)*	7 (4)*
0 (control for leaf or bark)	-	44.9; 45.1; 44.3; 45.3	55.9; 53.9; 53.7; 55.7	80.9; 80.8; 80.9; 80.4	-	-
50	Leaf	36.0; 35.2; 34.4; 35.1	43.2; 43.1; 43.0; 43.3	60.8; 58.9; 60.6; 60.0	69.7; 70.8	-
	Bark	43.6; 41.7; 40.0; 40.5	45.4; 45.4; 45.2; 45.3	64.8; 65.1; 65.4; 64.9	79.6; 74.8	-
200	Leaf	29.8; 29.1; 28.7; 29.1	40.3; 40.2; 40.6; 41.1	51.0; 50.5; 49.7; 50.5	68.3; 68.8	-
	Bark	34.4; 34.8; 34.2; 35.1	40.6; 40.6; 39.7; 40.6	57.0; 57.9; 57.1; 57.3	75.6; 75.2; 77.4	-
800	Leaf	26.5; 26.0; 25.3; 26.0	38.5; 37.4; 38.5; 38.6	50.2; 49.5; 50.3; 49.7	64.8	82.2; 80.1
	Bark	32.6; 32.5; 34.8; 36.2	38.5; 38.3; 37.4; 38.9	54.1; 55.1; 54.2; 53.8	69.0; 65.7; 65.2	84.4

* Values in parenthesis () represent days after initial treatment.

Appendix C4

Quantitative Expression of Drug Efficacy

(Survival Index, I_S)

The survival index (I_S) can be used instead of probit analysis to compare drug efficacy. It was used by Nathan et al (1979) to express degrees of life prolongation in the absence of cure. The expression is given by the formula:-

$$I_S = \frac{\overline{Nt} - \overline{Nu}}{\overline{Nt} + \overline{Nu}} = \frac{\frac{\Sigma nTn}{\Sigma Tn} - \frac{\Sigma nUn}{\Sigma Un}}{\frac{\Sigma nTn}{\Sigma Tn} + \frac{\Sigma nUn}{\Sigma Un}}$$

where:

Is = survival index;

$\overline{Nt}$ = average survival time (in days) for treated animals;

$\overline{Nu}$ = average survival time (days) for untreated controls;

Tn = the number of treated animals surviving for n days after challenge; and

Un = number of untreated controls surviving n days.

The numerator of the expression (the difference between the average survival time of treated animals and untreated controls gives a reasonable measure of drug effect. Using this formula, Nathan et al (1979) obtained a useful normalised index which ranges from -1 to +1. When the prolongation of life due to the drug is very great and survival of controls is insignificant by comparison, I_S is close to +1. Alternatively, when the difference in mean survival with or without drug is negligible, I_S is approximately zero. If a drug is toxic and the treated animals die on the average much sooner than the controls, I_S approaches the value -1.

Appendix C5

Extrapolation of Human Dosage Regimen from the Animal Study

Minimum concentration of aq. extract that elicited reasonable prophylactic and curative effects in the mice

= 200 mg/Kg

Average weight of an adult (human) = 60 Kg

∴ Amount of extract needed to be consumed by an adult

= 60 x 200 mg

= 12000 mg = 12 gm.

However, 50 tablets of leaf powder on extraction yielded

= 3.864 gm

Hence one tablet would contain $= \frac{3.864}{50}$ gm

= 77.28 mg

An adult of average 60 Kg weight will therefore, consume

$= \frac{12000 \text{ mg}}{77.28 \text{ mg}}$ tablets

= 155.28 tablets.

This implies that an adult human being requires approximately, 155 tablets, each of 300 mg weigth formulated from *Azadirachta indica* leaves.

Using the same process, an adult will require approximately 621 tablets of a 300 mg tablet weigth from an effective therapeutically effective drug concentration of 800 mg/Kg (a concentration that effected more than 75% suppression of parasitaemia in the mice).