Standardisation and Tablet Formulation of the Extracts of the Seeds of *Glinus lotoides*

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Dedicated to my parents, my wife and my son

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ACRONYMS

DAB	Deutsche Arzimettle Buch, (German Pharmacopoeia)
DSC	Differential scanning calorimetry
ELISA	Enzyme-linked immunosorbent assay
GAS	Gravimetric sorption analyser
GC	Gas chromatography
HPLC	High pressure liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
NMR	Nuclear magnetic resonance
RH	Relative humidity
RIA	Radioimmunoassay
rpm	revolution per minute
TBAs	Traditional birth attendants
TGA	Thermal gravimetric analysis
TLC	Thin layer chromatography

SUMMARY

Glinus lotoides Linne, locally known as "*Mettere*" is an annual herb, the seeds of which are traditionally used as anthelminthic for the prevalent tape worm infection. The taenicidal activity has been attributed to the saponins present in the seeds.

This study reports on the development of suitable extraction and purification methods of the seeds of the plant; the development of analytical methods for quantification of the saponins in the crude extracts; the physico-chemical characterisation of the extracts; and tablet formulation thereof.

Extraction and purification methods to obtain "total saponins" of *G. lotoides* were developed and the purified extract (i.e., total saponins) was used as a standard for quantitative determination. Sensitive colourimetric and UV-spectrophotometric methods were developed and β-escin, a mixture of triterpenoidic saponins obtained from *Aesculus hippocastanum*, was used as a control standard.

For the colourimetric assay method, the official German Pharmacopoeia (DAB) method of determination of β-escin was adopted with slight modification. In this, saponins were reacted with ferric chloride in acetic acid: sulphuric acid (1:1) and the red-coloured complex formed was measured at 540 and 409 nm for β-escin and saponins of *G. lotoides* respectively. Linear regression of the calibration curves yielded equation $Y = 6.6314X - 0.0279$ ($r = 0.9983$) for β-escin and $Y = 12.669X + 0.0456$ ($r = 0.9993$) for saponins of *G. lotoides* at concentrations ranging from 0.02 to 0.12 mg/ml and 0.01 to 0.06 mg/ml, respectively.

Pre-derivatization of saponins with 10% acetic acid in 0.1 N HCl rendered them UV-active and absorption was measured at 238 nm. Linear regression of the calibration curve yielded an equation of Y = 7.9514X - 0.0536 (r = 0.9983) at concentrations ranging from 0.02 to 0.12 mg/ml. Both methods showed comparable and reproducible results on identical samples and were employed for standardisation of the extracts of the seeds of *G. lotoides*.

Physico-chemical properties of the powdered extracts of the seeds such as particle size and distribution, morphology, water sorption pattern, densities, flow properties and compaction profiles were investigated. Based on the density results, properties, namely, porosity, consolidation index (Carr's index) and Hausner ratio were calculated.

The effects of some extraction processes, such as extracting solvents and methods of extract drying, on the physical properties of the extracts were investigated and a suitable solvent system and drying method were determined.

Based up on the results of the preformulation studies, the most suitable extract of the seeds of *G. lotoides* (570 mg of Extract A) was formulated and compressed into 1.15g oblong tablets (having long and short diameters of 20.1 and 8.2 mm respectively). The effects of types of filler/binder, disintegrants and compression force were investigated. Tablet properties such as hardness, disintegration time and friability were evaluated and suitable tablet formulation was developed.

1. INTRODUCTION

1.1 Traditional herbal medicine

Therapeutic use of plants for the treatment of human illnesses dates back to time immemorial, and humans have been using herbs and other natural components to develop medicinal compositions to help the sick amongst them. Through the years, several herbs have been proven to be safe and effective for treating certain disease conditions. Evidence of effectiveness of herbs in the diagnosis, cure and prevention of disease states exists in every culture throughout the world and it has been mentioned in the Bible (1).

Plants that are used as medicines have been referred to as herbs. Originally, the term herb only applied to non-woody plants. Nowadays, herb refers to any part of any plant used for flavouring or medicine. Although the term herb can also be equated with food spices, colouring and dyeing substances, it is generally used in reference to any plant, or any part of a plant, having nutritional and/or medicinal value(s). Hence, herb can be a fruit, a bark, a flower, a leaf, a root, or a whole plant (1).

Historically, the most important uses of herbs were their medicinal values. For most of his existence, man had various but limited resources for treating injuries and diseases. Separately and in combination, any of the following have been utilised: magic and sorcery, prayer, music, crude operations (amputation, bleeding, trepanning), psychotherapy, physical therapy (diet, rest, exercise, fresh aid, water), and internal and external remedies prepared from plants, animals, and minerals. Of all these, plant remedies represent the most continuous and universal form of treatments. In all these, plants were the basic source of therapeutic products for professional and folk medicine from the earliest days until the twentieth century. Folk medicine, the household use of

simple herbal remedies, is based on word-of-mouth tradition that probably stretches in an unbroken line to prehistoric times (2).

Today traditional medicine, characterised by the use of herbs and other natural products, is a regular component of health care in many countries such as China, Japan, India, France, Germany, several countries of South America, Egypt, etc. In a number of European countries, herbal medicines are regarded legally as any other manufactured pharmaceutical drugs. Health professionals in these countries (including medical doctors, pharmacists and nurses) are taught about herbal medicines as part of their college education (3).

In order to appreciate the progress and development made in the use of herbal medicines and to understand the prevailing scenario, a brief historical background is presented.

1.1.1 Historical review

Prehistoric man used plants to treat physical complaints, as he used them for food and shelter, long before written history began. He undoubtedly learned by instinct and by generations of trial and error that certain plants were useful for treating illness, just as he learned that some were good to eat and others could cause poisoning and death. In the written record, the study of herbs dates back over 5,000 years to the Sumerians, who described well-established medicinal uses for such plants as laurel, caraway and thyme. The first known Chinese herbal book, dating back to about 2700 BC, lists 365 medicinal plants and their uses including "ma-huang", the shrub that introduced the drug ephedrine to modern medicine. The Egyptians around 1000 BC are known to have

used garlic, opium, castor oil, coriander, mint, indigo, and other herbs for medicine, food, and dyes. The Old Testament has also mentioned the use and cultivation of herbs (4).

Hippocrates advocated the use of a few simple herbal drugs along with fresh air, rest, and proper diet to help the body's own "life force" in eliminating the problem. Galen, on the other hand, believed that direct intervention with large doses of more or less complicated drug mixtures, including plant, animal, and mineral ingredients, often accompanied by some magical incantations, was necessary to correct bodily imbalances that caused disease. The first European treatise on the properties and uses of medicinal plants, "De Materia Medica", was compiled by the Greek physician Dioscorides in the first century A. D. His compendium of more than 500 plants remained an authoritative reference into the seventeenth century. Similarly important for herbalists and botanists of later centuries was the Greek book that founded the science of botany, "Theophrastus' Historia Plantarum", written in the fourth century BC (4).

The uses of plants for medicine and other purposes changed little during the Middle Ages. The early Christian church discouraged the formal practice of medicine, preferring faith healing; but many Greek and Roman writings on medicine, as on other subjects, were preserved by diligent hand copying of manuscripts in monasteries. The monasteries thus tended to become local centres of medical knowledge, and their herb gardens provided the raw materials for simple treatment of common disorders. At the same time, folk medicine in the home and village continued uninterrupted, supporting numerous wandering and settled herbalists. In the eleventh century, medical schools began to return teaching Galen's system. During the Middle Ages, then, a herb with a

reputation for healing might find itself prescribed by a peasant grandmother, sold by a wandering herbalist, charmed as an ingredient in a magic potion or amulet by a wise-woman or compounded into a complex and often vile mixture to be dispensed by a physician in the hope that it would drive out whatever possessed the victim. Above all, plants were burdened with a mass of both pagan and Christian superstition that often was more important than their actual properties (5).

The continuing importance of herbs for the centuries following the Middle Ages is indicated by the hundreds of herbals published after the invention of printing in the fifteenth century. "Theophrastus' Historia Plantarum" was one of the first books to be printed, and "Dioscorides' De Materia Medica" was not far behind (4).

The fifteenth, sixteenth, and seventeenth centuries were the "great age" of herbals, many of them available for the first time in English and other languages rather than Latin or Greek. The first herbal book to be published in English was the anonymous "Grete Herball" of 1526, by two best-known herbalists in England.

The seventeenth century, however, also saw the beginning of slow erosion of the pre-eminent position held by plants as sources of therapeutic effects. The introduction by the physician Paracelsus of active chemical drugs (like arsenic, copper sulphate, iron, mercury, and sulphur), followed by the rapid development of chemistry and the other physical sciences in the eighteenth and nineteenth centuries, led inexorably to the dominance of chemotherapy (4). The day-to-day reality, however, changed little in the new millennium. Plants still provide the basic materials for medicine, dyeing, and perfume (2).

1.1.2 Traditional medicine in Ethiopia

The antiquity of Ethiopian traditional medicine could not be established with certainty due to lack of adequate historical resources. However, it is mentioned that, it has long been practised (6).

Traditional herbal medicines are believed to be the major means of health care and the only available resource for the majority of the population in the developing countries like Ethiopia. In rural societies, due to the lack of or inadequate modern health services and facilities, folk herbal preparations are still the dominant method of therapy for common ailments. These remedies are fairly well accepted, easily available and bear a minimal cost. Traditional medical practitioners of Ethiopia are categorised into various groups such as: Herbalist ("*Kitel betash*"), Herbal medicine dispensers ("*Medhanit atechi*"), Chirugions (bone settlers, tonsil cutters, tooth extractors, etc), "Spiritual healers", etc (6).

A preliminary study conducted on medicinal plant in Ethiopian market indicated that the majority of the Ethiopian population continue to rely on household remedies and the medicines of traditional healers (7). Although herbal medicines have been used by the Ethiopian people for centuries, they have not yet been developed to a level as to serve as a substitute or complementary to modern pharmaceutical drugs.

The economical problem of Ethiopia or any other developing country, is reflected in a number of ways, including health problems. One of the major contributing factors to the health problems in Ethiopia is the high cost and/or shortage of medicines. As a way of tackling this problem, more attention should be given to the development and

utilisation of herbal medicines. Therefore, other approaches have to be explored for developing herbal medicines and any advantages of traditional medicine have to be utilised.

1.1.3 Advantages of traditional medicine

In developing countries large number of people die daily of preventable or curable diseases due to the lack of even simple health care. Diseases in developing countries are often associated with malnutrition and, as a result, those that do survive often never recover completely from the effects. The socio-economic problems of developing countries such as: extremely limited resources, large population growth, poor communications, individual and community poverty, lack of education, high infant, maternal and childhood mortality, etc., make it difficult for conventional health services to reach the people. The quality of basic sanitation in most developing countries is also very much below the level considered necessary for the prevention and control of communicable diseases and the promotion and maintenance of good health. Hence, it is important to develop systems, in developing countries, through which effective health care can be made both accessible and acceptable to the people. Due to these social-economic statuses of developing countries, the magnitude of the health problem, and the few resource available, any advantages of traditional medicine can be and should be viewed (8).

The following can be considered as the major advantages of traditional medicine:

a) Traditional medicine is cheaper than modern medicine.

The cost of modern medicine is increased by modern health technology that in many cases it is inappropriate or irrelevant to the immediate needs of the people in

developing countries. Cost of modern medicine which relies heavily on institutions and professional staff, is increasing faster than that of simpler health programmes, such as the traditional or indigenous system of medicine available in Asia and China (8).

While drugs alone are not the only means of providing health care, they do play an important role in protecting, maintaining and restoring the health of people. The drug budget for many developing countries still represents a sizeable proportion of their total health expenditure. In developed countries drugs account for 10%–30% of the total cost of health care, while these figures rise to 30%–50% in many developing countries (8).

b) Traditional medicine is more accessible to most of the population.

Because of the shortage of health care facilities such as hospitals and health centres, and medical as well as paramedical staff needed for modern health care delivery systems, large proportion of the population of developing countries have to rely on traditional or indigenous forms of medicine

c) Traditional medicine enjoys a wider acceptability among the people of developing countries than does modern medicine.

This could be due partly to the inaccessibility of modern medicine as indicated above. However, the major contributory factor is the fact that traditional medicine blends readily into the socio-cultural life of the people in whose culture it is deeply rooted. It was the only form of health care available to such people before the advent of modern medicine and there are instances today in Ethiopia as well as other countries such as Guatemala, Kenya, Ghana and Nigeria, for example, where people who are close to hospitals consult traditional healers as a first choice (9).

Apart from socio-cultural factors the acceptance of traditional methods of therapy in a given community often depends on economic factors. Many patients can only afford the help of traditional medical practitioners although they are unable to determine whether this form of medical aid is harmless, dangerous, useful or useless. Others seek traditional medical help because of the community's belief that certain types of disease, especially psychological or mental cases, only respond to traditional cures (10).

d) Traditional medicine is a potential source of new drugs, a source of cheap starting products for the synthesis of known drugs, or a cheap source of known drugs such as resepine from *Rauwolfia* species, vinblastine from *Catharanthus roseus*.

Critical evaluation of the phytochemistry and pharmacology of alleged curative plants and the traditional pharmaceutical practices employed has been strongly recommended. Many new pharmaceutically active natural products, effective in treating an array of diseases and maladies including various tumors and HIV, have been reportedly isolated from different sources of vegetation, including the bark, roots, leaves, fungi and cell culture of many different species (11-13).

e) The practitioners of traditional medicine could serve as additional sources of health manpower in developing countries.

This is especially true where a developing country is trying to achieve total health coverage for its people. The traditional practitioners would, of course, need to be retrained, especially in simple hygiene, general modern health concepts, health education (including nutrition), elementary health care, environmental sanitation, epidemic diseases, emergencies and referrals and record-keeping. Many developing

countries have realised this and formalised training for traditional practitioners particularly of traditional birth attendants (TBAs) and conduct workshops aimed at retraining. Such training could enable the traditional practitioners to participate in primary health care, especially in maternal and child health, family planning, community immunisation and public health services. For example, in Niger, retraining of the TBAs in the Maradi Department was responsible for a significant lowering in the high maternal and child mortality. Their retraining took some 15 days and was essentially practical and related to all phases of childbirth, including pre-natal and post-natal care. Trained TBAs are able to perform more than the traditional rudimentary tasks of burying the placenta and giving the most elementary care to the child and the mother. The short training in a health centre maternity ward or a district rural hospital was achieved at a cost of US$ 21 per TBA, including the cost of equipment and supplies. Trained TBAs compensate for the scarcity of maternity hospitals and contribute to achieve the objectives of reduced infant mortality, improve standards of hygiene during delivery, education of the mother in nutrition during pregnancy and weaning and recording birth (14).

In general, traditional medical practitioners can be useful to health care and their incorporation offers the following advantages (8):

i) They can fill the vacuum in the health care created by the shortage of manpower and the high cost of training modern health workers;
ii) They have developed traditional skills in the dispensing of curative, preventive, and rehabilitative care;

iii) In their treatment techniques, they use an astute approach to human ecology and health;

iv) They belong to the same culture as their patients; sharing common believes, values and symbols of communication;

v) They are effective in some aspects of psychosomatic medicine and in the use of local herbs for symptomatic treatment;

vi) They have skill in interpersonal relations, including counselling with sympathy, identification, and concern and

vii) They are unhampered by inadequate transportation in rural areas.

However, the incorporation of the traditional medicine into the heath care system prior to systematic investigation and standardisation, may cause problems. Hence, the possible limitations and disadvantages of traditional medicine in these respects are mentioned below.

1.1.4 Limitations of traditional medicine

a) Lack of standardisation and control

Herbal remedies are becoming increasingly popular among patients as treatment for different medical problems. However, there are many negative effects of herbal preparations due to lack of standardisation and adequate control in the process of formulation that contribute to contamination and toxicity of herbal products (14-17). Moreover, most of the traditional herbal preparations are polypharmaceuticals containing more than one plant materials. It has been reported that the "Shengxue Syrup" is composed of 20 Chinese medicinal herbs and used for invigorating the function of spleen (18).

b) Lack of proof for safety and efficacy

Perhaps one of the greatest arguments against traditional medicine is the lack of scientific proof of its efficacy. Most of the claims are made by the traditional medical practitioners themselves and many have not been thoroughly investigated scientifically. Hundreds of herbal products and homeopathic remedies are available to the consumer, but most of these have not been proved safe or effective (19). Patients who self-medicate with herbs for preventive and therapeutic purposes may assume that these products are safe because they are "natural," but some products cause adverse effects. *Strychnos* species containing strychnine and eserine from *physostigma venenosum* are common examples.

Little is known about phytomedicine safety. There has been an increase in the number of herbal side effects reported in the literatures. Many cases, however, could have gone unreported because herbal medicine is usually self-prescribed and often ignored by health practitioners during the patient's care. Identifying adverse effects is further hindered because it is not always possible to assess the quality of certain herbal medicinal products (19).

c) Questionable diagnosis

Another shortcoming of traditional medicine is the imprecise diagnosis often given by the practitioners. The imprecision is due to the fact that the pathology of certain diseases is not known to the traditional medical practitioner. As a result they tend to treat the symptom rather than the disease, which can sometimes lead to further complications (8).

d) Inaccurate dosage and interaction risk with modern drugs

Another criticism levied against traditional practitioners is that their medicaments lack precise dosage. The potential risk of herbal medicine interacting with a prescribed drug is also a concern with the increased use of phytomedicines. Recently, several interactions have drawn the attention of the medical community. An interaction between ginseng, one of the most popular herbs with multiple health claims, and warfarin, a drug with numerous well-recognised drug-drug interactions has been reported (20). A randomised, crossover drug-herbal interaction study was conducted in 22 healthy volunteers to investigate the effect of three major Chinese herbal medicines containing 60 to 66 mg of glycyrrhizin on the pharmacokinetics of prednisolone. The effect of the three glycyrrhizin preparations on the pharmacokinetics of prednisolone differs even though they contain a similar amounts (21).

In general, most of the problems of traditional medicine are associated with a lack of standardisation and proper formulation. Formulation of traditional herbs into modern phytopharmaceuticals shall provide solution to most of these limitations of traditional medicine.

1.2 Phytopharmaceuticals

Phytopharmaceuticals also called, phytomedicinals or phytomedicine are herbal preparations made from different parts of herbs. They are prepared in different formulations and dosage forms including tablet, capsule, elixir, powder, extract, tincture, cream and parenteral preparations. Herbal products can be crude plant materials or extracts, which contain substances as active ingredients. They do not normally contain synthetic compounds, and are different from traditional herbal

preparations in several aspects. A single isolate or active principle derived from plants such as digoxin or reserpine tablets is not considered herbal medicine. Phytopharmaceuticals are not to be confused with homeopathic preparations. Homeopathic medicine, founded in the eighteenth century by the German physician Samuel Hahnemann, also uses herbs and other natural products, but it is based upon the "law of similar" and the "law of dilution" (22,23).

Thousands of herbal products are available world wide. In some countries, phytopharmaceuticals are perceived as drugs and can be bought over the counter or prescribed by physicians. In the United States, herbal preparations are not classified as drugs by the Food and Drug Administration (FDA). They are classified as "dietary supplements" with a few exceptions such as Capsicum, Elm Bark, Juniper, Karaya Gum, Psyllium and Witch Hazel. In Germany, results of pharmacological research, clinical studies and bibliographic searches must be submitted to a special commission of the German Ministry of Health, Commission E, for risk-benefit evaluation before marketing authorisation is granted. In France, the sale of herbal medicines is under less stringent but more pragmatic regulations; an herb is considered a safe and effective drug if it can be "traditionally" proven safe and effective. In a number of other European countries, herbal medicines are regarded legally as any other manufactured pharmaceutical drugs. These medicinal products are included in official pharmacopoeias of many of the European countries and publications of the European Economic Community. Accordingly, the criteria for the evaluation of the safety and the efficacy apply to herbal remedies as they do to other products (23,24).

Many pharmaceutical companies are involved in the manufacturing of herbal medicines following the standard procedures set for modern drugs. The products are available in various dosage forms and they are dispensed in pharmacies and drugstores along with other pharmaceutical products. Health professionals in these countries (including medical doctors, pharmacists and nurses) are taught about herbal medicines as part of their college education, and many physicians prescribe them for their patients like any other official medicines (22).

Although herbal medicines have been used by the Ethiopian people for centuries, they have not yet been developed to a level as to serve as a substitute or complementary to pharmaceutical drugs. Research efforts directed towards this goal appear to focus primarily on the collection and identification of traditional herbal medicines and laboratory investigation of the medicinal properties of some of these plants. While this approach can obviously be beneficial in the long-run, it does not seem to have an impact on the current health problem in the country. Therefore, another approach for developing our herbal medicines needs to be explored. A strategy for successful research work in the development of phytopharmaceuticals, selection of herbs (prioritisation) and an overview of production techniques is discussed below.

1.2.1 Guidelines for research work in formulation of herbal drugs

Research work in the field of medicinal herbs must be multidisciplinary in approach and objectives must be set for the team. The following objectives can be useful guides (25, 26):

a) Identify health conditions which can be treated by traditional drugs as, or even more, effectively than modern drugs, taking into consideration the pathophysiological and psychosomatic aspects of the symptom;
b) Identify the agents, either medicinal plants or other natural substances, which can be used to produce useful traditional drugs;
c) Standardise the drugs and their raw materials (essentially medicinal plants). Standardisation should be based upon activity, quality, safety, etc;
d) Develop appropriate technology for production in the following areas: selection; collection; cultivation; conservation; and processing to form the most suitable dosage forms for distribution, storage and administration.

Herbal drug formulation is a challenge in pharmaceutical technology due to the complex physicochemical properties of its multicomponent materials. Potential instabilities of the pharmacologically active and coactive substances as well as incompatibilities and interactions of the extracted compounds and excipients have to be considered. Microbial contamination of the applied plant material might limit the shelf life of the products. Nowadays, besides the traditional preparations, stable aqueous or hydroethanolic preparations (syrups, juices, drops, liniments), preparations from partially dried extracts (fluid, or semisolid: gels, ointments and creams), or solid dosage forms (tablets, coated tablets as well as soft and hard gelatine capsules) of the extracts are in use. In addition, the compliance to these formulations have been enhanced due to simplified application and improved sensorial quality (27). There is a tremendous range of sophistication in the processing of herbal formulations from crude herb material or from standardised extracts. Nonetheless, there are some common stages. An overview of these processes such as: preparation of the herbal extracts, standardisation,

preformulation (characterisation) and formulation of phytopharmaceuticals as well as the machines that perform these functions are discussed as follows.

1.2.2 Preparation of herbal extracts

1.2.2.1 Collection and/or harvesting

A decision has to be made as to whether medicinal plant is collected from wild or harvested from cultivated sources, or both. When plants are collected form their natural habitat, they are said to be "wild-crafted" and when they are grown utilising commercial farming techniques, they are said to be "cultivated". If the plant grows readily in the wild and produces a good yield of active constituents, or takes several years to mature, then collection of wild plants is the most practicable. However, cultivation of medicinal plants is desirable under the following conditions (28):

a) When too few of the plants grow wild,
b) When the wild source is sparsely distributed,
c) When the wild plants are geographically inaccessible,
d) When there is need to improve the yield of active principles produced by the plants growing wild. Cultivation can produce more plant growth and hence a better yield, by introduction of good agricultural practices, good soil and pest control, etc,
e) When there is governmental control over the plants, for example plants such as cannabis. Plants yielding dangerous drugs or addictive drugs are best cultivated under license,
f) When only a desired species or variety of a particular plant is to be used for preparing galenicals. Collection of plants from cultivated sources ensures that the plant collected is the one that is desired. When a herb is collected from wild, there

is a much greater chance that a wrong herb will be picked, a situation that could lead to serious consequences, and

g) Cultivation can allow a better and quicker post-harvest treatment of the plant drug, such as drying and extraction.

The collectors of herbs may vary from uneducated natives and self-proclaimed "herbalists" to skilled botanists of professional training. The use of analytical techniques can be employed to guarantee that the plant collected is the one desired. The mode of harvesting also varies from hand labour to very sophisticated equipment. The mode is not as important as the time of harvesting. A plant should be harvested when the part of the plant being used contains the highest possible level of active compounds. Again, this is ensured by the use of proper analytical techniques (8).

There are many ways to grow, gather, and harvest herbs. Herbs are considered the "best" by some practitioners when they are naturally grown in the wild, untouched by industrial pollutants. Others prefer herbs that are cultivated indoors, away from all contaminants, in a controlled environment. Some herbalist's recommend gathering only certain herbs (depending on the seasons, the weather, and the time of day) to achieve the highest level of medicinal qualities. And still others may disregard this practice, and will purposefully plant herbs 'out of season' so that they will be available for sale year round (8).

In addition to growth and gathering techniques, harvesting practices vary as well. Recommendations may include taking the whole plant at once (buds, roots, seeds,

leaves and blooms), or taking each part of the plant in a particular order, and only using younger, or older, plants.

1.2.2.2 Drying of the plant material

Immediately after harvesting or collection, i.e. after separation of certain parts of the plant or after the whole plant, including roots, has been dug up, processes commence which mostly become externally apparent with the withering of the plants. Storage organs and preservable parts of plants such as tubers, seeds and certain dried fruits in principle undergo the same phenomena. However, the modifying processes proceed slower because of the low water content and as the structure of the skin of seeds and some fruits prevent evaporation (29).

After harvesting, most herbs have moisture content of 60%-80%, and cannot be stored without drying. Otherwise, important compounds would break down, or micro-organisms would contaminate the material. During drying, the plant material must not be damaged, or suffer losses, that would prevent it from conforming to accepted composition standards. With proper drying, the herb's moisture content will be reduced to less than 14%. Most of the enzymes in the plant need adequate water to act. The water in dry drugs is so low that decomposition reactions do not usually occur or it is retarded to such an extent that a certain degree of storage stability is guaranteed (30).

The majority of herbs require relatively mild conditions for drying. Commercially, most plants are dried within a temperature range of 100-140° F (i.e., 38-60° C). During the process of drying, enormous physical and chemical changes takes place in the interior cells of the herb. The gel state of the plasma is distributed by dehydration and

the active substances originally dissolved in the cell fluid partially precipitate and are attracted back into the structurally altered plasma. The enzymes predominantly localised in the plasma come in contact with the active substances. In some plants the active substances and the enzymes exist in different, spatially separated cells. During harvesting and subsequent drying process, the tissue will be destroyed and the enzyme and the active principle will come in contact and react (31).

There are two ways of protecting the drug from the action of enzymes. These are i) drying the plant material as soon as possible and ii) denaturing the enzymes. The latter is known as stabilisation of the active principle and it is hardly practised on large scales (30).

1.2.2.3 Garbling

Garbling refers to the separation of that portion of the plant to be used from other parts of the plant, dirt, and other extraneous matter. This step is often done during the collection process. Although there are machines that perform garbling, usually garbling is performed by hand. Unwanted materials such as plant debris, soil, discoloured flowers or diseased leaves that could increase the bulk and cause adulteration should be excluded (30).

1.2.2.4 Grinding and classification (sorting)

Grinding or comminution a herb means, mechanically breaking down either leaves, roots, seeds, or other parts of a plant into very small units ranging from larger coarse fragments to fine powder. Grinding is employed in the production of crude herbal products as well as in the initial phase of extraction. Classification means separation of

the particles of the plant material into various classes according to their physical characteristics, usually particle size (30).

The purpose of comminution and classification of drugs is to obtain certain fractions with similar particle size distribution and to avoid large amount of dusts. Clean, narrow, dust-free fraction is of great importance for further processing and subsequent extraction. Excess dust, for example, results in clogging of percolators and cerates difficulties in filtration and clarification. Large difference in the particle size is also undesirable as the menstrum takes longer time for complete extraction of the coarser materials. On the other hand, finely shredded fractions are desirable for the preparation of teabags and phytopharmaceutical sachets (30).

During grinding, the material is often pre-chopped, or minced, before feeding it into the machine to enhance the grinding process. Comminution process can be seen as an energetically inefficient operation since only about 1% of the total energy achieves the comminution. The rest, 99% is wasted (30).

In the process of grinding, a number of machines can be used, but the most widely used is the hammer mill. These machines are robust and simple in design. The hammers, arranged radially, follow the rotation of the shaft to which they are attached, breaking up the material that is fed into the machine from above. On the walls of the chamber is a grid or sieve, which determines the size of the material that is passed through it. Sifter or classifier mills combine a grinder with a sifter or classifier in one apparatus. The material is put in the mill, the sifter wheel removes the fine material. The coarse material remains in the milling chamber until it has reached the final desired degree of

fineness. Other types of grinders include knife mills (cutting mills), teeth mills (toothed disc mills), cross beater mills (disintegrators), blower mills (micronisers), pin mills, fluted roller grinders and cold mills. Choice of a mill for the comminution of a drug plant material is determined both by the properties of the starting material and by the purpose for which the milled product is subsequently to be used (30).

1.2.2.5 Extraction

The process of extraction is used in making tinctures, fluid extracts, and solid extracts. Extraction, in the context of phytopharmaceuticals, refers to separating by physical or chemical means the desired material from a plant with the aid of a solvent. Most extracts in phytopharmaceuticals utilise water or hydroalcohol as solvents to remove the soluble compounds from the herb. The exceptions are liposterolic extracts, which are produced either through the use of lipophilic solvents or with the aid of hypercritical carbon dioxide (30,32).

Extraction processes for drugs can be divided into two major groups, processes that result in the establishment of a concentration equilibrium between solution and solid residue and those where the drug is extracted exhaustively. In the former method, the extraction process comes to an end when the concentration gradient between miscella (solution containing extracted substance) and residue becomes zero. That is, when the distribution of the extracted substance between miscella and drug residue reaches a certain equilibrium value (k) (30).

$$k = \frac{\text{Concentration of the extracted substance in the miscella}}{\text{Concentration of the extractable substance in drug residue}} \quad (1.1)$$

Numerous factors, such as the degree of comminution, quality and nature of the starting material, moisture content of the plant material, volume and selectivity of the solvent system, mixing ratio, imbibition of the drug, temperature, pH, possible interaction between dissolved constituents and structural materials (usually, adsorption), etc., could affect the value and the time required to reach the equilibrium of extraction (30). Extraction methods such as maceration (simple maceration, kinetic maceration, remaceration and digestion (maceration at higher temperature, usually 40–50° C)), vortical (turbo) extraction (extraction using high-speed mixers or homogenisers), ultrasound extraction (using sound waves to accelerate extraction) and extraction using electric energy are some examples of this group (30).

The second processes are those where the drug is extracted exhaustively. Exhaustive extraction is defined as the complete removal of the desired extractive substances from the drug material. Extraction methods such as simple percolation, repercolation and continuous counter current extraction come under this categories (30).

In the extraction of drugs from plant materials, two extraction processes take place simultaneously. These are: i) rinsing of the extractive substances out of disintegrated plant cells and ii) dissolution of extractive substances out of intact plant cells by diffusion.

The latter requires the prior steeping and swelling of the drug plant material in order to increase the permeability of the cell walls. This step can be analysed in three stages: i) penetration of the solvent into the plant cells and swelling of the cells; ii) dissolution of

the extractive substances, and iii) diffusion of the dissolved extractive substances out of the plant cells.

Substances are dissolved more rapidly from disintegrated cells than from intact cells. A greater degree of comminution of the drug plant material results in a larger proportion of disintegrated cells and hence more rapid dissolution. Therefore, finely divided drugs would be preferable as long as it doesn't affect the subsequent separation and filtration processes (33).

1.2.2.6 Concentration

After extraction of the herb, the resulting solutions can be concentrated into fluid extracts or solid extracts. Large manufacturing operations utilise techniques and machines, such as thin layer evaporators, tube vaporisers with auto-circulation or pumped circulation, falling film vaporisers and centrifugal rotary vaporisers, that ensure the extracted plant components are not damaged. These machines work by evaporating the solvent, thus leaving the plant compounds behind. The solvent vapours pass into a condenser whereby they return to a liquid state, and can then be reused. The result is separation of the extracted materials from the solvent such that the final product is a pure extract, and the solvent can be used again and again (30).

1.2.2.7 Extract drying

Although there are still a number of liquid forms of extracts on the market, namely tinctures, fluid extracts, and soft extracts, the preferred industry method is to dry the extract to a solid form. The main reasons are greater chemical stability and reduced cost. Alcohol, a solvent for tinctures, for example is often more expensive than the herb

itself. Tinctures are usually concentrates. In 1:5 tincture, for example, 80% of the bottle is alcohol and water, and only 20% herbal material. In essence, the cost of the alcohol is a major portion of the retail price of the tinctures. Hence, tinctures are not as cost effective, nor they are as stable, as dry powdered extracts. Tinctures, fluid extracts, and soft extracts are also easily contaminated by bacteria and other micro-organisms. Liquid forms of extracts also promote chemical reactions, which may tend to break down the herbal compounds.

Number of drying techniques and apparatus are employed in herbal processing industries, including freeze dryers, spray dryers, vacuum dryers, roller dryers and microwave dryers (34).

Freeze drying, frequently termed 'lyophilization,' is a drying process where the solvent, normally water, is first frozen and then removed by sublimation in a vacuum environment. In the pharmaceutical industry, the aqueous solution is filled into suitable containers, which are loaded onto temperature-controlled shelves installed in a large drying chamber. The temperature of the shelves is lowered to about -40° C and the solution is transformed into a solid (i.e., ice and solid solute). The drug and excipients may or may not crystallise, depending on the nature of the formulation and details of the freezing process. After complete solidification (i.e., after crystallisation of ice and solutes or after crystallisation of ice and formation of glassy solutes), the system is evacuated by vacuum pumps, and the shelf temperature is increased to initiate the ice sublimation stage of the process, denoted as "primary drying." Water vapour created by sublimation is transported through the partially dried product "cake" and is condensed on low-temperature (about -60° C) surfaces in the condenser chamber. The rate of

primary drying increases greatly as the product temperature increases, but each formulation has a maximum temperature that cannot be exceeded without damage to the product. Even after all ice has been removed by sublimation, the product typically contains large amounts of water approximately 20%-50% "dissolved" in the amorphous portion of the solid. Removal of most (or essentially all) of this dissolved water is accomplished during the secondary drying stage of the process. Secondary drying is normally carried out at elevated product temperature to achieve efficient water removal (35).

In general, a product is freeze dried if the aqueous solution does not have sufficient stability for marketing and if the product cannot be crystallised in bulk. Compared to spray drying, freeze-drying is a low-temperature process, and is normally regarded a being less destructive to the product, particularly for proteins. In addition, control of sterility and foreign particulate matter is relatively straightforward. Thus, although inherently expensive from the viewpoints of plant cost and energy consumption, freeze-drying is often the processing method of choice for the production of a heat sensitive product (35).

The impact of high costs of freeze-drying is lessened by the concern for purity and the realisation that raw material costs frequently dwarf the freeze drying costs. Moreover, an understanding of the freeze-drying process coupled with process optimisation studies can significantly reduce the duration, and therefore costs, of the process as well as ensure control of product quality (35). The dried powdered extract is then standardised and then formulated into suitable dosage forms.

1.2.3 Standardisation of extracts

Standardised extracts (also known as "guaranteed potency" extracts) refer to an extract guaranteed to contain a "standardised" level of active compounds. Stating the content of active compounds (rather than the concentration ratio) allows for more accurate dosages to be made by manufacturers and practitioners.

The best scenario for determining the quality of herb is the level of active components or key biological markers. Regardless of the form that the herb is in, it should be analysed to ensure that it contains these isolated key components at an acceptable standardised level. More accurate dosages can then be given for maximum beneficial effect (36).

The effectiveness of any herb or herbal product from a pharmacological perspective is dependent upon providing an effective dosage of the active compounds. Regardless of the form of the herbal preparation, clinical effectiveness requires efficient delivery of an active dosage within the body. Standardisation for the level of active components, or key biological markers, is the only real means to assure an appropriate delivery of an effective dosage for the user (37).

Over the past years, recognised world authorities on botanical alternative medicine have defined, and established, specific standards of excellence for herbal extracts. The use of herbal medicine is widespread and growing. Because many herbal medicines have significant pharmacological activity, and thus potential adverse effects and drug interactions, health care professionals must be familiar with this therapeutic modality. Therapies outside the medical mainstream tend to suffer from a dearth of research and

critical evaluation. Critics, supporters and alike note the conceptual and practical difficulties in studying many complementary and alternative therapies such as acupuncture, homeopathy, and meditation. Herbal medicine, however, lends itself well to standard evaluation methods (38).

The objective of analysing and standardising crude extracts and their preparations is to develop reliable methods, which will be used to correlate the active ingredients in the plant to their therapeutic activities. To achieve this objective it is necessary, (a) to know precisely which chemicals present are the active ones, (b) to be certain that the final analytical figure obtained represents these chemicals quantitatively and exclusively; and (c) that the analytical process is sufficiently defined so that all workers using it will get the same figure for the same sample (36).

1.2.3.1 Needs for standardisation

When a herbal product is not standardised, it is impossible to know how much of the herb's key compound(s) are actually present in it or even the product is the claimed one. Moreover, it is difficult to assure that the level of the active ingredient is the same in all the products. Only with proper standardisation, that those doubt and problems will be solved.

In some parts of the world where no government standards and quality control are enforced, adulteration and contamination pose safety as well as efficacy problems. The presence of adulterants such as arsenic, mercury, lead or prescription drugs in herbal medications can cause significant toxicity. In china, awareness of this public health concern was developed after finding that 23.7% of traditional Chinese medicines (total

of 2,609 samples) collected over a year among eight hospitals in Taiwan were spiked with adulterants such as caffeine, acetaminophen, indomethacin, hydrochlorothiazide and other "undeclared" prescription drugs (39,40).

Standardised extracts are also needed to carry out reliable clinical trials. The standardisation of an extract does not include only a whole of analytical controls: it requires also a thorough description of the starting drug and of the whole extraction process, both basic items for the constancy of the quality of an extract. Another, but not minor aspect in the standardisation of an extract is its nomenclature which must include all the necessary data for a clear definition (drug, physical state, solvent of extraction, composition) of the extract itself (41).

1.2.3.2 Assay development

In order to make all future quantitative measurements in formulation and quality control an analytical technique has to be developed. Establishing an assay method is a basis for all other activities that will be carried out to develop a standard dosage form. Success in design of a standard dosage form usually depends on the assay method developed.

Raw materials that are used for the preparation of various dosage forms should be of acceptable standards. If the material is of pharmacopoeial standard, then assay development may not be of greater concern. On the other hand, for new materials which are not of pharmacopoeial standard such as new phytopharmaceutical extracts, the assay has to be developed (26).

In the past, it was often difficult to determine the quality of the product produced since many of the active principles of the herbs were scientifically unknown. However, recent advances in extraction processes, coupled with improved analytical methods have reduced this problem of quality control. The concentration method of expressing the strength of an extract does not accurately measure potency since there may be great variation among manufacturing techniques and raw materials. By using a high quality herb (a herb high in active compounds), it is possible to have a more potent dried herb, tincture, or fluid extract compared to the solid extract that was made from a lower quality herb. Standardisation is the solution to this problem (25,26).

Improvements in analytical methods have definitely led to improvements in harvesting schedules, cultivation techniques, storage, activity, stability of active compounds, and product purity. All of these gains have resulted in tremendous improvements in the quality of herbal preparations now available. For example, optimal activity and quality collection should be done at a time when the active ingredient is present in the greatest amount. Improvements in analysis have led to more precise harvesting of many herbs. Methods currently utilised in evaluating herbs and their extracts include: organoleptic, microscopic, physical, chemical/physical and biological (25,26).

Organoleptic means the "impression of the organs." Organoleptic analysis involves the application of sight, odour, taste, touch, and occasionally even sound, to identify the plant. Obviously, the initial sight of the plant or extract is so specific that it tends to identify itself. If this is not enough, perhaps the plant or extract has a characteristic odour or taste. Organoleptic analysis represents the simplest, yet the most human, form of analysis (25,26).

Microscopic evaluation is indispensable in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs, and in detection of adulterants (e.g. insects, animal faeces, mould, fungi, etc.) as well as identifying the plant by characteristic tissue features. Every plant possesses a characteristic tissue structure, which can be demonstrated through study of tissue arrangement, cell walls, and configuration when properly mounted in stains, reagents, and media (25,26).

In crude plant evaluation, physical methods often are used to determine the solubility, specific gravity, melting point, water content, degree of fibre elasticity, and other physical characteristics of the herb material. Various chemical/physical methods also are used to determine the percentage of active principles such as saponins, alkaloids, flavonoids, enzymes, vitamins, essential oils, fats, carbohydrates, protein, ash, acid-insoluble ash, or crude fibre present (25,26).

The final analytical process requires more precise assays to determine quality. Sophisticated techniques, such as high pressure liquid chromatography (HPLC) and nuclear magnetic resonance (NMR), are often used to separate and identify molecules. The readings from these machines provide a chemical "fingerprint" as to the nature of chemicals contained in the plant or extract. These techniques are invaluable in the effort to properly and fully identify herbs, as well as standardised extracts. The plant or extract can then be evaluated by various biological methods to determine pharmacological activity, potency, and toxicity (25,26).

1.2.3.3 Quality control of herbal extract

Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. Regardless of the form of herbal preparation, some

degree of quality control should exist. Without proper quality control, there is no assurance that the herb contained in the dosage forms is the same as what is stated on the label. The widespread disregard for quality control in the health food industry has tarnished the reputation of many important medicinal herbs (25,26).

The solution to most of the problems is for manufacturers and suppliers of herbal products to adhere to quality control standards and good manufacturing practices. With improvements in the identification of plants by laboratory analysis, consumers should at least be guaranteed that the right plant is being used. Consumers, health food stores, pharmacists, and physicians who use or sell herbal products should ask for information from the suppliers of herbal products on their quality control processes. As more consumers, retailers, and professionals begin asking for quality control from the suppliers, it is possible more quality control processes will be utilised by manufacturers. Numerous reports are found in the literature dealing with development of quality standards and quality control of herbal preparations (42-46).

1.2.4 Preformulation studies

Before embarking upon formulation, it is usually required to carry out preformulation studies with regard to physical and chemical characteristics of the material to be formulated (crude plant material, extracts and excipients) (26).

1.2.4.1 Characterisation of the extract

Preformulation studies of the extract include both physical and chemical characteristics of extracts. The physical properties of the extract include: appearance (colour), odour, solubility in formulation solvents, pH in solution and distribution coefficient,

flowability and compressibility, bulk and tapped densities, total solids and ash content. The chemical characterisation, on the other hand, includes phytochemical identification of the chemical groups such as saponins, alkaloids, etc (26).

1.2.4.2 Stability studies

Stability studies of extracts are important in determination of shelf-life of the product. Any pharmaceutical dosage forms, whenever possible, must possess desired effects and stability for specified time (shelf-life) without loss of potency or producing undesirable effect. The stability studies conducted on the extract also assist in selection of suitable excipients and packaging materials for the subsequent formulation. To achieve these objectives, pharmaceutical technologists have to assess the extracts and all preparations under various kinds of experimental conditions including the accelerated and normal storage conditions (26).

Dry extracts create less stability problems than soft or liquid extracts. However, almost in all dry extracts there will be some free moisture that may be contributed by the active ingredients or secondary components. The major stability problem of dry extracts is, therefore, their hygroscopicity. Most of the enzymes in the plant need adequate water to act. The water content in dry drugs is so low that decomposition reactions do not usually occur or are highly reduced. Many enzymes survive the drying process and are reactivated when water or moisture is once again available. The relative humidity (RH) of the storage condition, therefore, highly affects the stability of the product (26).

Stability problems in solution are very significant. The two most serious stability problems in solutions are hydrolysis and decomposition due to microbial attack. A great number of decompositions are frequently observed in solutions, particularly in aqueous solutions. Therefore, it is necessary to know some properties, for instance, the pH at which the extract becomes stable (26).

1.2.4.3 Compatibility studies

Every dosage form contains one or more excipients. Interaction between the extract and excipients may adversely affect the safety and efficacy of the dosage forms. Successful formulation of a stable and effective dosage form depends on appropriate selection of excipients. Any physicochemical interactions between the extract and the excipients have to be investigated. Confirmation of compatibility can be ascertained by Thin Layer Chromatography (TLC), Differential Scanning Calorimetry (DSC) or other analytical methods (26).

1.2.5 Formulation studies

The aims of formulation, in the light of bioavailability considerations, are to produce a drug delivery system such that a unit dose contains the intended quantity of a drug and is usually released in order to achieve the required onset, intensity and duration of clinical response (26).

There are several ways to dispense herbs. The most common methods are herbal pastes, juices, decoctions, hot or cold infusions, powders, tablets, capsules, aromatics, tinctures or extracts (alcohol or glycerol bases), liniments, syrups, poultices and fomentations, medicated oils, salves and ointments, lotions, teas, and whole herbs. Each type is good

for specific ailments, and often may be used to take full advantage of the healing attributes of each (26,30).

1.2.5.1 Factors to be considered in formulation of traditional herbs

The following factors have to be considered in formulation of traditional herbal drug (26).

a) Determination of the most appropriate extract based on the physicochemical properties;
b) Determination of the administration route;
c) Determination of the most likely dosage form;
d) Appropriate selection of excipients;
e) Aspects of preparation processes and suitable packaging, and
f) Therapeutic considerations of the dosage form.

1.2.5.2 Standardisation of dosage forms

Standardisation of traditional medicine does not mean the standardisation of the finished products alone. In modern pharmaceutical formulations, the quality control department does control the quality of the raw materials, intermediate products and the finished products. There is also in-process quality control at various critical stages of the production processes. The same is true in standardisation of traditional medicine. Standardisation of traditional medicine may be divided into two major parts: Standardisation before dosage formulation and standardisation after dosage formulation (26).

Standardisation before dosage formulation will be done mainly by phytochemists/ pharmacognosists and includes all processes required for preparation of extract, while standardisation after dosage formulation is done primarily by the pharmaceutical technologist (30).

1.2.5.3 Types of possible herbal preparations

Almost all dosage forms can be prepared from crude herbal extracts. However, the solid and liquid dosage forms are the most commonly used to prepare dried or fresh extracts (30).

Tablets

Oral administration is the dominant method of delivering drugs for systemic absorption because of its safety and simplicity. Thus, a great interest has been focused so far on the design of oral dosage forms with optimal therapeutic properties.

For economical reasons as well as for the convenience of the patient, the compressed tablet is the most widely used dosage form. Almost all tablets can be prepared from extracts. Extracts are expected to be in dry state. Dry extracts are usually hygroscope and may create problems in formulation. These problems can be minimised or avoided by granulation of the extract with non-aqueous adhesive solution or using high porosity excipients such as silica gel (26).

Capsules

The processing of plant extracts into soft gelatine capsules does not usually present any particular difficulties. Most extracts, despite being hydrophilic, can easily be converted

into pumpable suspensions with lipophilic dispersant when they are finely powdered. In contrast, direct filling into hard gelatine capsules is made difficult by the poor flow properties of most dried extracts. Hard gelatine capsules are suitable for non-hygroscopic and freely flowing extracts or granules (30).

Oral solutions

Any forms of the extract (dry, liquid or soft) can be formulated into an oral solution using suitable solvents. In some cases, additional co-solvents may be used. Oral solutions commonly used in formulation of traditional herbal drugs are tinctures, fluid extracts and syrups. Suspensions and emulsions are also employed to formulate water insoluble extracts and medicinal oils, respectively (26,30).

Dosage forms for local use

Dermatological and rectal ointments preparations are available from plant extracts to treat different skin conditions and haemorrhoid. Ointment and liquid dosage forms can be used in such cases (26).

1.2.5.4 Bioavailability of dosage forms

The term bioavailability is used to express the rate and extent of absorption from a drug delivery system into the systemic circulation. To produce a systemic effect, the drug must be released form the dosage form and absorbed. Before any drug product is released to market for consumption the bioavailability must be assessed. In formulation of traditional herbs, bioavailability may be assessed by determination of the induced clinical response or by measuring a certain guide substance uniformly available in the extracts. The former method is used when measuring and quantifying the extract chemically is difficult. However, a number of attempts have been made to measure

bioavailability by correlating the activity to certain compound. The type of dosage form, properties of the excipient(s) and method of preparation usually affects the bioavailability of the active ingredient (47-49).

1.2.5.5 Stability testing of dosage forms

Drug stability, in addition to being of paramount importance to product shelf-life, can also affect bioavailability. A drug product must possess the desired clinical effects for specified time without loss of potency and producing undesirable effect. Methods for assay of formulation have to be developed in order to assess the stability of dosage forms. Packaging and storage conditions should also prevent deterioration and loss of potency. Proper selection of packaging material improves stability of the formulation (26).

1.3 *Glinus lotoides* Linne

Glinus lotoides Linne, locally known as "*Mettere*" is an annual herb growing in low land dry areas. It is found mostly in tropical and subtropical region such as Ethiopia, Sudan, Uganda, Egypt, India, Pakistan and South America. As it is found in different countries its traditional uses also differ. In India and Pakistan, for instance, the plant is used as antifungal agent, and for fishing (50). In Ethiopia, the seeds of *G. lotoides* is traditionally used as anthelminthic for the prevalent tape worm infection.

There are well over 25 plants in Ethiopia, which are traditionally used against the widely prevalent tapeworm parasite (6,7). However, literature survey reveals that most of them are well known to be toxic (51-53). Since *G. lotoides* is claimed to have little or no side effect, it enjoys a considerable reputation as a taenicide in the Ethiopian traditional medicine. The traditional mode of administration of *G. lotoides* is to orally

consume about 20 grams of the whole powdered seeds as suspension in '*tella*', a traditional local beer or covered with some food (e.g., banana) to make it palatable. This amount is believed to remove the tapeworms completely from the intestine of the patient (7,54-56).

The *in vitro* taenicidal activity of *G. lotoides* on *Taenia saginata* worms (54) and the *in vitro* and *in vivo* taenicidal activity on *Hymenolepis nana* worms have been reported (55,56). The ethanolic extract of the seeds of the plant has been shown to be highly active *in vitro* against *H. nana* worms with an approximate 50% effective dose (ED_{50}) of 0.003 mg/ml. It has also been shown that a concentration of 1 mg per ml of the extract exhibited comparable *in vitro* taenicidal activity with 0.05 and 0.01 mg/ml of niclosamide and praziquantel respectively (55).

The *in vivo* anthelminthic activity of the extract has been determined in Albino mice infested with *H. nana* worms. The extract has been found to be active against *H. nana* worms when given both as a single dose as well as multiple doses. The extract, however, has been found more effective when administered as multiple doses than as single dose. In single dose treatment, higher concentration of the extract (as much as 3.3 g per kg, i.e., 100 mg per 30 g mouse) was required to expel all the worms. Whereas the total multiple dose that cured the mice completely amounted to only 0.6 g per kg (i.e., 3 mg a day for 6 days per 30 g mouse). In the control experiments, a single dose of praziquantel (0.6 mg per mouse) resulted in 100% cure within 48 hrs while a single dose of niclosamide (0.5 mg) had only 73.3% efficacy (59).

The taenicidal activity of *G. lotoides* has been attributed to saponins present in it (54-56). The cestocidal activities of saponins obtained from various plants have been documented (57,58). Preliminary pharmacological and toxicological studies on the extract of *G. lotoides* indicate that the extract does not affect the blood pressure, heart rate or the ECG of anaesthetised rabbits, bile production in guinea pigs, or contraction of frog isolated heart after oral administration (59,60).

The hydroalcoholic extract of the seeds of the plant has also been found to be orally non-lethal in doses as large as 100 mg per 30g mouse, which is equivalent to 3.3 g per kg. The 50% Lethal Dose (LD_{50}) of the extract has been reported to be around 4.5 g per kg (150 mg per 30 g mouse) (56).

Modern anthelminthic drugs such as praziquantel and niclosamide have been widely used in Ethiopia for Hymenolepiasis treatment and praziquantel is a drug of choice. However, although praziquantel is rapidly taken by helminths leading to a massive contraction and paralysis of the helminth's musculature, it is also absorbed to the systemic circulation of the patient causing a number of side effects. Furthermore, praziquantel is excreted in breast milk in concentrations ranging up to 25% of those found in the maternal serum. Nursing mothers, therefore, should stop breast-feeding when beginning treatment with praziquantel and breast-feeding should not be resumed until 72 hours after treatment is completed. During this time, the breast milk should be expressed and discarded. The biotransformation of praziquantel is metabolism in liver. Therefore, the risk-benefit should be considered when moderate to sever liver disease exist (61).

In view of such shortcomings and side-effects of praziquantel, *G. lotoides* may be a potential candidate and an alternative drug for the treatment of tapeworm infection. The extracts of *G. lotoides* are believed to be free from systemic side-effects since saponins are not significantly absorbed from the gastro-intestinal tract (62).

1.3.1 Classification

G. lotoides L. is classified under a family molluginocea and order centrospermea. The order centrospermea contains 13 families, one of which is molluginocea. The family mollugnacea contains about 16 genera and 100 species. The family molluginocea was often included within the family Aizoaceae, but now it is generally accepted that the 16 genera of mollugnacea are not related to the other genera of Aizoaceae. The genus Glinus contains different species (63).

G. lotoides was known by different botanical synonyms such as *Glinus dicatamoides, Glinus setiforous, Mollugo gliuns, Mollugo hirta,* and *Mollugo lotoides*.

1.3.2 Description

G. lotoides L. is an annual or short-lived perennial prostrate herb which is widely distributed throughout the highlands and the banks of rivers and lakes of Ethiopia. The taxonomic feature of the plant (Fig 1.1), shows that it is glabrous or densely short hairy. The leaves are opposite or clustered at nods and apparently verticillate, narrowly elliptic to subround. The plant contains stipules and the flowers are bisexual and clustered in axillary. Detailed description of the plant is given elsewhere (63).

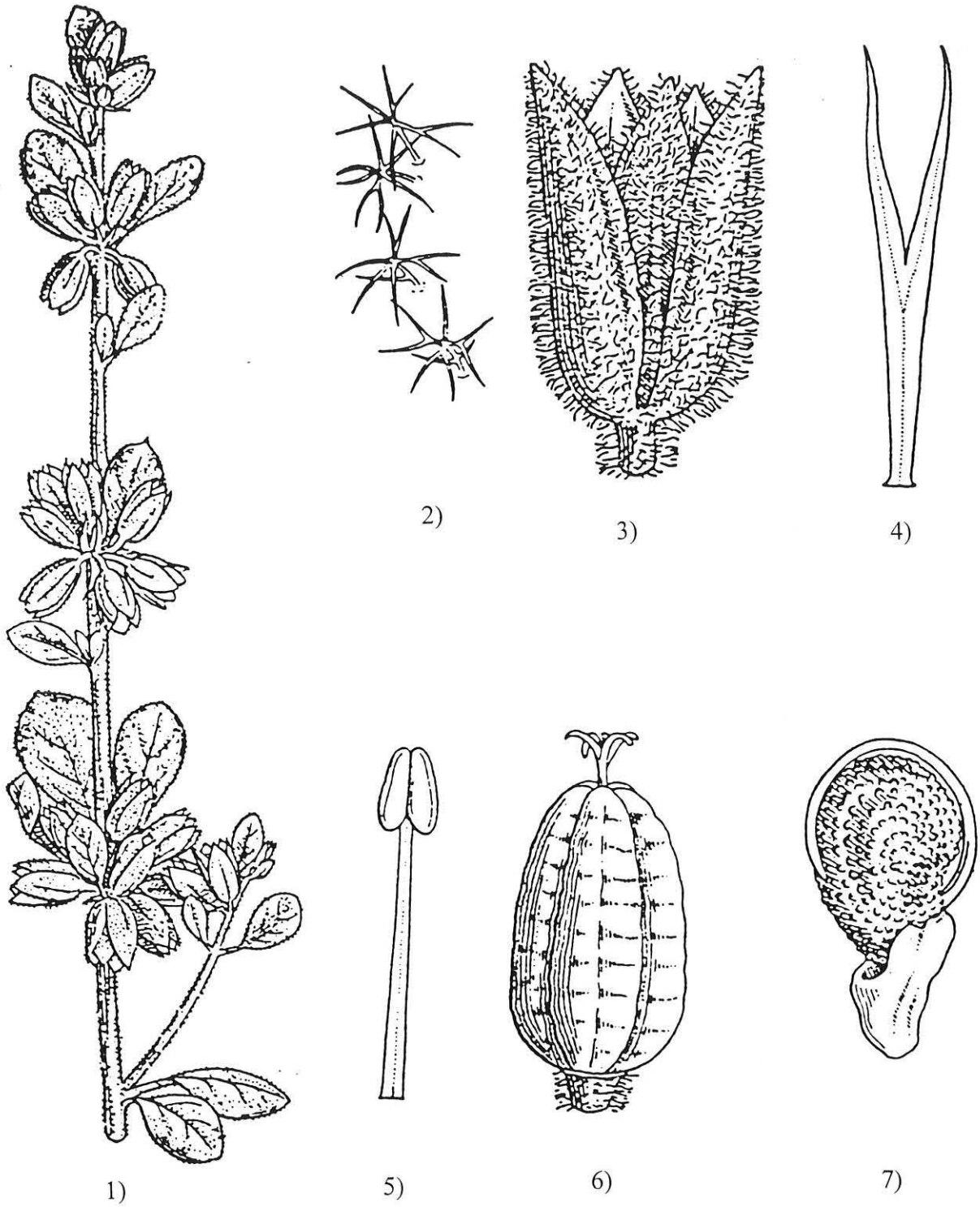


Figure 1.1: Pictorial representation of the plant *G. lotoides*. [1) habit; 2) hairs form leaf; 3) flower; 4) staminode; 5) stamen; 6) ovary; 7) seed].

1.3.3 Chemical constituents

The seeds of *G. lotoides* contain 10% crude saponins (56) and 14% oil (64). The etherial composition of the plant contains fatty acids, glycosides of sitosterols, stigmasterol, flavonoids and waxes (64). Abegaz *et al* have isolated biologically active triterpene glycoside from the seeds of the plant (65). The chemical structure of this saponin is shown in Figure 1.2. Acid hydrolysis of the saponin with 8% methanolic HCl yielded oleanolic acid, D-glucose and L-arabinose and the ratio of glucose to arabinose has been found to be 2:1.

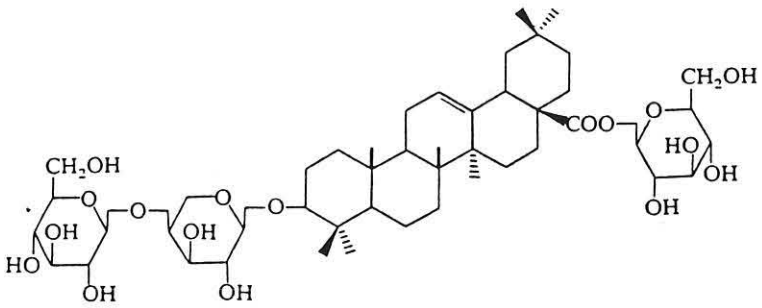
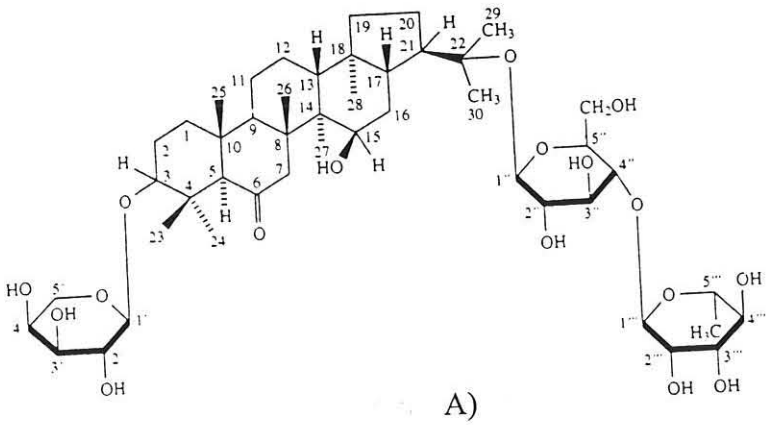


Figure 1.2: Chemical structure of a saponin from the seeds of *G. lotoides* var. *linne*.

Five triterpenoidal saponins (Glinusides A, B, C, D and E) have been isolated from the n-butanol fraction of *G. lotoides* var. *dicatamnoides* (66,67). Chemical structures of those saponins, which were determined by spectral analysis are shown in Figure 1.3.



A)

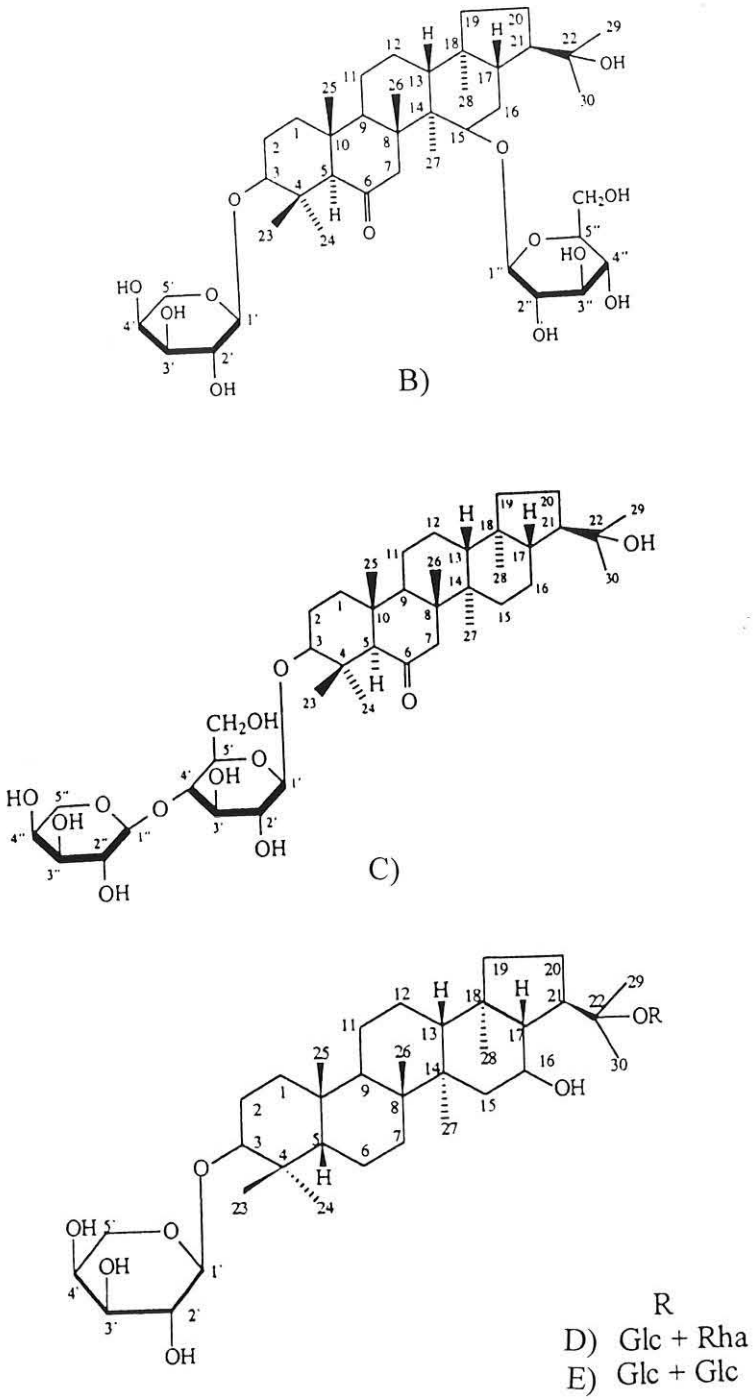


Figure 1.3: Chemical structure of saponins (Glinusides) from the n-butanol fraction of *G. lotoides* var. *dicatamnoides*.

[A) 3-*O*-*β*-L-arabinopyranosyl-22-*O*-*β*-D-glucopyranosyl(4←1)-α-L-rhamnopyranosyl-15*β*-hydroxyhopan-6-one; B) 3-*O*-*β*-L-arabinopyranosyl-15-*O*-*β*-D-glucopyranosyl-22*β*-hydroxyhopan-6-one; C) 3-*O*-*β*-D-glucopyranosyl (4←1)-*β*-L-arabinopyranosyl-22*β*-hydroxyhopan-6-one; D) 3-O-*β*-L-arabinopyranosyl- 22-α-L-rhamnopyranosyl (1→4)-*β* -D-glucopyranosyl-5-*β* -H-16-*β* -hydroxyhopane; E) 3-O-*β*-L-arabinopyranosyl-22-O-*β* -D-glucopyranosyl-(1→4)-*β* -D-glucopyranosyl-5 -H-16-*β* -hydroxyhopane]

1.3.4 Methods of saponin analysis in plant extracts

An important aspect of the modern use of plant extracts as pharmaceutical preparations is the characterisation and determination of the individual active constituents. Such is also the case for saponin preparations, which often requires sophisticated techniques for the isolation, structure elucidation and analysis of their component, triterpene and steroid glycosides.

Various analytical methods have been employed for the qualitative and quantitative determination of saponins: such as haemolysis, piscicidal activity, gravimetry, spectrophotometry, TLC, Gas Chromatography (GC), HPLC, etc. (68,69). A very simple but inadequate test is based on the foam-forming properties of saponins.

Qualitative and quantitative determination of saponins based on their classical properties, such as: haemolysis, surface activity, fish toxicity, have largely been replaced by photometric methods such as densitometry, colourimetry of derivatives, GC and HPLC. For example, the quantitative analysis of *Ginseng radix* which is official in the Pharmacopoeia Helvetica VII, relies on reaction with glacial acid/sulphuric acid and spectrophotometry at 520 nm of the red colour formed (70). ß-escin, component of horse chestnut (*Aesculus hippocastanum*, Hippocastanaceae), saponin can be spectrophotometrically determined after treatment with a mixture of ferric chloride, acetic acid and sulphuric acid. This method is official in German Pharmacopoeia (DAB) (71,72).

Spectrophotometric methods are very sensitive but not suitable for estimating saponins in crude plant extracts since the reactions are not specific and coloured products may be

formed with compounds which accompany the saponins, such as phytosterols and flavonoids (70). Another problem, common to much of the analytical work on saponins, is their incomplete extraction from the vegetable material which leads to a high variation between batches (71).

Various analytical methods are usually recommended in the literature for the analysis of saponins, including colourimetry, TLC, liquid chromatography-mass spectrometry (LC-MS), HPLC, radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) (71).

The principle of colourimetry method is the formation of colour using suitable colour-forming agent(s) and measurement at specific wavelength. Various colour-forming agents commonly used for colourimetric measurement of saponins include: ferric or cerium salts, such as ferric chloride in acetic acid and sulphuric acid, or cerium (IV) sulphate in inorganic acids, (sulphuric acid); aromatic aldehydes; Libermann-Burchard reagent; antimony (III) chloride in acetic anhydride-acetic acid or in nitrobenzene-methanol; Ehrlich reagent and carbazole (71).

1.4 Objectives of the present work

The following are the objectives of this research work:

1.4.1 General Objectives

a) Standardisation of the extracts of the seeds of *G. lotoides*, and

b) Formulation and evaluation of the extracts into tablets.

1.4.2 Specific Objectives

a) Development of suitable and efficient extraction method and validation of the extraction processes;

b) Extraction and purification of the extract to obtain relatively purified saponins of *G. lotoides* which could serve as reference standard;

c) Development of analytical methods for quantitative determination of saponins in crude extracts of the seeds of *G. lotoides*;

d) Physico-chemical characterisation (preformulation studies) of the crude extracts of the seeds of *G. lotoides*, and

e) Formulation and evaluation of tablets from the extracts.

2. EXPERIMENTAL

2.1 Materials and Methods

2.1.1 Materials

Fruits of *G. lotoides* were purchased from the local market, '*Merkato*' in Addis Ababa. β-escin was obtained from Merck, Darmstadt, Germany.

Hexane, methanol, ethanol, n-butanol, diethyl ether, chloroform, 1-propanol, 1,2 dichloroethane, ethyl acetate, 2-butanone, toluene and formic acid, ferric chloride, hydrochloric acid, glacial acetic acid, sulphuric acid, vanillin and p-anisaldehyde, potassium chloride, sodium chloride, sodium nitrite, sodium bromide ($NaBr.2H_2O$), potassium carbonate (K_2CO_3 $1.5H_2O$), magnesium chloride ($MgCl_2.6H_2O$) and silicon dioxide anti-foam emulsion were all of pharmaceutical grade.

Four types of direct compression filler/binders (coded as filler/binder A, B, C and D), two disintegrants (disintegrant A and B), glidant A and lubricant A were all of pharmaceutical grade.

Precoated silica gel 60 F 254 20X20 cm aluminium sheets were purchased from Merck, Darmstadt. Hydranal® Composite 2 (one component reagent for volumetric Karl Fisher titration) and sodium tartarate dihydrate were from Riedel-deHaën, AG, Seelze. Formamide was obtained from Merck, Darmstadt. The above mentioned companies are all in Germany.

All materials were used as received.

2.1.2 Methods

2.1.2.1 Extraction and purification of saponins from the seeds of *G. lotoides*

2.1.2.1.1 Defatting and determination of total fat content

The cover pods of *G. lotoides* were separated from the fruits and the seeds were powdered. 5 g of the powdered seeds were defatted three times with 50 ml of n-hexane using Ultura-Turrax mixer (T25, Janke & Kunkel, GmbH & Co. KG, IKA®-Labortechnik, Staufen i. Breisgau, Germany) at 9500 rpm for 10 min. The extract was filtered, the solvent was removed under reduced pressure and the amount of fat removed was determined. Total fat content was determined by defatting 5 g of the seeds of *G. lotoides* with 100 ml of n-hexane five times.

2.1.2.1.2 Extraction with 80% methanol

5 g of dried defatted seeds were extracted with 50 ml of 80% methanol three times successively using ultura-turrax mixer at 9500 rpm for 10 min. The aqueous methanol extracts were filtered, combined and concentrated in rota vapour (under reduced pressure) at 40° C. The concentrated extract, i.e., the remaining aqueous part, was then dried using freeze dryer (LYOVAC GT2, FINN-AQUA®, GmbH, Hürth, Germany) or vacuum oven dryer (40° C and – 0.9 Bar).

Freeze Drying

The concentrated extract was first cooled to –70° C for 24 hrs in a separate deep-freezer (GFL®, Gesellschaft für Labortechnik mbh, Burgwedel, Germany) and then dried in a freeze drier for 48 hrs using the following heating programme: –30° C for 5 hrs; -25° C for 5 hrs; -20° C for 5 hrs; -15° C for 5 hrs; -10° C for 4 hrs; -5° C for 8 hrs; 0° C for 6

hrs; 10° C for 5 hrs and 20° C for 5 hrs. The freeze dried extracts were kept in a desicator over activated silica gel.

Vacuum oven drying

The concentrated extract was dried to a semi-solid mass in forced air oven (Typ: ULE 700, Memmert GmbH, Schwabach, Germany) and then dried over night in a vacuum oven at 40° C and – 0.9 Bar.

2.1.2.1.3 Fractionation of the dried extract

0.5 g of the freeze or vacuum oven dried extract was dissolved in 30 ml of water and mixed with 30 ml of water saturated with n-butanol in a separatory funnel. The mixture was shaken vigorously for two minutes and allowed to separate. On separation the butanol layer (i.e., upper phase) was taken and dried under reduced pressure. The dried extract obtained was weighed and referred to as "n-butanol fraction".

2.1.2.1.4 Purification of n-butanol fraction

0.1 gram of the dried n-butanol fraction was dissolved in small volume of methanol (i. e., 2 ml) and was added into large volume of diethyl ether (≈ 40 ml). The precipitate formed was separated by centrifugation (Megafuge 1.0R, Heraeus Instrumes, Osterode, Germany) at 2500 rpm, for 15 min at 5° C and dried. The purified extract obtained is referred to as "total saponins" of *G. lotoides.*

2.1.2.2 Colourimetric assay method of saponins of *G. lotoides*

2.1.2.2.1 Preparation of standard solution

30 mg of the purified extract (total saponins) were shaken in a flask with 20 ml of 0.1 N HCl for 15 min and filtered. The flask and the filter paper were rinsed twice with 5 ml

of 0.1 N HCl. The filtrates were combined and mixed with 20 ml of 1-propanol and 50 ml of chloroform in a 250 ml separatory funnel. The mixture was thoroughly mixed for 2 min and allowed to separate. On separation, the lower phase (i.e., chloroform-rich phase) was taken and the upper phase remaining in the separatory funnel was once again mixed with the lower phase of a ternary mixture of 30 ml 0.1 N HCl, 20 ml of 1-propanol and 50 ml of chloroform. The procedure was repeated three times. The three lower phases were combined, concentrated under reduced pressure at 40° C and dried in the vacuum oven. The dried product was washed with 10 ml of ether two times and dried. The final dry product obtained was then dissolved with 50 ml of glacial acetic acid in volumetric flask (72).

2.1.2.2.2 Preparation of control standard (ß-escin) solution

Similarly, 30 mg of ß-escin were dissolved in a flask with 30 ml of 0.1 N HCl for 15 min and mixed with 20 ml of 1-propanol and 50 ml of chloroform in a 250 ml separatory funnel. The mixture was thoroughly mixed for 2 min and allowed to separate. On separation the lower phase was taken and the upper phase remaining in the separatory funnel was extracted twice with the lower phase of a ternary mixture of 30 ml 0.1 N HCl, 20 ml of 1-propanol and 50 ml of chloroform. The lower phases were combined, concentrated and dried. The dried product was washed with 10 ml of ether two times and dried. The dry product obtained was then dissolved with 50 ml of glacial acetic acid in volumetric flask (72).

2.1.2.2.3 Preparation of colour forming reagent

Ferric chloride-acetic acid reagent is an official reagent for colourimetric determination in German Pharmacopoeia (DAB). In this, 75 mg of ferric chloride were dissolved in

50 ml of glacial acetic acid. The solution was then added into 50 ml of concentrated sulphuric acid previously cooled in an ice bath. The colour-forming reagent was always freshly prepared (72).

2.1.2.2.4 Formation of coloured complex

One ml of the standard or control standard solutions was mixed with 4 ml of ferric chloride-acetic acid reagent in a tightly fitting reagent flask. The mixture was then heated over a water bath at 60° C for 25 min with occasional shaking. After 25 min the solution was immediately cooled to a room temperature under running water (72).

2.1.2.2.5 Measurement of absorption spectra

The absorption spectra of the coloured solutions (i.e., total saponins of *G. lotoides* or β-escin–ferric chloride acetic/sulphuric acid mixtures) were measured over range of wave lengths from 190 to 900 nm using computerised spectrophotometer (Perkin-Elmer GmbH, UV/Vis Spectrometer Lambda 16, Überlingen, Germany). A mixture of 1 ml of acetic acid and 4 ml of ferric chloride acetic acid reagent, which is then heated at 60° C for 25 min, was used as a blank solution (72).

2.1.2.2.6 Preparation of standard curves

Six different concentrations of saponins of *G. lotoides* (0.01–0.06 mg/ml) and β-escin (0.02–0.12 mg/ml) ferric chloride complex were prepared and the absorbances were measured at wave lengths of 409, 460 and 540 nm. The experiments were repeated six times and calibration curves were drawn. The confidence intervals (simple and 10 times), the correlation coefficient and the residual points were determined. Linear

regression equation was obtained and was used in the calculations of saponin concentrations.

2.1.2.2.7 Analysis of saponins in the crude extracts

50 mg of the hydromethanolic crude extract of the seeds of *G. lotoides* were shaken in ultrasonic water bath with 40 ml of 0.1 N HCl for 15 min and the solution was filtered. The residue in the flask and on the filter paper was washed twice with 10 ml of 0.1 N HCl. The filtrates were combined and mixed with 40 ml of 1-propanol and 100 ml of chloroform in a separatory funnel. On separation, the lower phase was taken and the upper phase remaining in the separatory funnel was mixed with the lower phase of a ternary mixture of 30 ml 0.1 N HCl, 20 ml of 1-propanol and 50 ml of chloroform two times. The lower phases were combined, concentrated under reduced pressure at 40° C and dried in vacuum oven. The dried product obtained was washed with 10 ml of ether two times, dried and dissolved with 50 ml of glacial acetic acid.

The solution was then quantitatively mixed with ferric chloride acetic acid reagent and heated over a water bath at 60° C for 25 min. Upon the formation of stable colour, the absorbance was measured at 409 nm and the concentration was calculated.

2.1.2.2.8 Effect of heating time on the colour formation

The effect of heating time on reaction of saponins of *G. lotoides* with ferric chloride was evaluated by measuring the absorbance of 0.05 mg/ml saponins of *G. lotoides*-ferric chloride mixture at various time intervals 0, 5, 10, 15, 20, 25, 30 and 35 mins. Time zero was obtained by measuring the absorbance without heating and the temperature was kept constant at 60° C.

2.1.2.3 UV-Spectrophotomeric analysis method of saponins of *G. lotoides*

2.1.2.3.1 Preparation of standard and test solutions

0.2 g of saponins of *G. lotoides* or β-escin was dissolved in 100 ml of glacial acetic acid in a volumetric flask. Similarly, appropriate amount of the crude extract was dissolved in glacial acetic acid to obtain test solution

2.1.2.3.2 Absorption spectrum of the standard solutions

1 ml of the standard solutions were mixed with nine ml of 0.1 N hydrochloric acid and the absorbance spectrum of the solution was recorded over a wave length range of 190–900 nm. 10% acetic acid in 0.1 N HCl was used as a blank solution.

2.1.2.3.3 Preparation of calibration curves

Six different concentrations (0.02–0.12 mg/ml) were prepared by mixing 0.1–0.6 ml aliquot of the standard solutions with nine ml of 0.1 N HCl and adjusting the volume to 10 ml with glacial acetic acid. The absorbance of the solution was recorded at 238 nm. The experiments were repeated six times and calibration curves were drawn. The confidence intervals (simple and 10 times), the correlation coefficient and the residual points were determined. Linear regression equation was obtained and used in the calculations of saponin concentrations.

2.1.2.4 TLC-densitometry

2.1.2.4.1 Preparation of test and standard solutions

Test solution: 100 mg of the purified extract (total saponins) of the seeds were dissolved in 10 ml of 70% methanol.

Standard solution: 100 mg of β-escin were dissolved in 10 ml of 70% methanol (72).

2.1.2.4.2 Selection of suitable mobile phase

The following solvent systems were tested for their separation ability of the saponins of *G. lotoides.*

a) Upper phase of glacial acetic acid/water/n-butanol (10:40:50 v/v). This solvent system is official for β-escin in DAB 1999 (71).

b) Lower phase of the mixture of chloroform/methanol/acetic acid/water (63:32:12:18 v/v)

c) Chloroform/methanol/water/formic acid (50:40:8:1 v/v)

d) 1,2 dichloroethane/ethanol/methanol/water (50:20:20:6 v/v)

e) Ethyl acetate/methanol/water (85:17:13 v/v)

f) Ethyl acetate/2-butanone/water/formic acid (50:30:20:10 v/v)

g) Toluene/ethyl acetate/formic acid (40:10:5 v/v)

2.1.2.4.3 Preparation of TLC-detection reagents

P-anisaldehyde reagent

0.5 ml of anisaldehyde was mixed with 10 ml of glacial acetic acid, followed by the addition of 85 ml of methanol and then 5 ml of concentrated sulphuric acid.

1% vanillin ethanolic solution

1 g vanillin was dissolved in 100 ml ethanol. 95 ml of this solution was then mixed with 5 ml of concentrated sulphuric acid.

2.1.2.4.4 TLC development

Precoated silica gel 60 F 254 20X20 cm aluminium sheets was used as adsorbent. 5 µl of test and standard solutions were applied 15 mm from the lower edge and 10 mm from the sides of the TLC plate using calibrated disposable capillary tubes. After evaporation of the solvent, the plates were placed in solvent saturated chromatographic

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tank ensuring that they are vertical as much as possible and were developed. The mobile phase was allowed to run a distance of 12 cm. Upon completion, the TLC plates were dried using hot air, and spots were developed by immersion (for 10 sec) in 1% vanillin ethanolic solution or sprayed with p-anisaldehyde detection reagent. The plates were then heated at 120° C for 10 min.

For saturation of the chromatographic tank, the inner wall of the tank was lined with filter paper and a sufficient quantity of the mobile phase was added so that after impregnation of the filter paper a layer of appropriate depth obtained. The lid of the chromatographic tank was then replaced and allowed to stand for about 1 hour. The amount of solvent used is related to the dimension of the TLC plate used. All the TLC runs were conducted in a saturated tank as mentioned above.

2.1.2.4.5 Densitometric measurement

Developed TLC plates were allowed to stand for 30 min at room temperature and the density of the spots were scanned with densitometer CD 60 (Desaga, GmbH, Wiesloch, Germany).

2.1.2.5 Standardisation of the extraction method

2.1.2.5.1 Determination of solvent system for a maximum yield

Different concentrations of methanol in water (i.e., from 0 to 100% methanol) were examined for their extracting power of the various extracts. The amount of the crude extract, the n-Butanol fraction and the purified extract obtained was determined as described above. Anti-foam emulsion and centrifugation at 2500 rpm for 4 min were

utilised for solvent systems containing 50% methanol and below during ultura-turrax extraction and liquid-liquid extraction with n-butanol respectively.

2.1.2.5.2 Measurement of saponin contents in the crude extracts

50 mg of the crude extracts (0-100% MeOH) of the seeds of *G. lotoides* were shaken in ultrasonic water bath with 40 ml of 0.1 N HCl for 15 min and the solution was filtered. The residues in the flask and on the filter paper were washed twice with 10 ml of 0.1 N HCl. The filtrates were combined and mixed with 40 ml of 1-propanol and 100 ml of chloroform in a separatory funnel. On separation, the lower phase was taken and the upper phase remaining in the separatory funnel was mixed two times with the lower phase of a ternary mixture of 30 ml 0.1 N HCl, 20 ml of 1-propanol and 50 ml of chloroform. The lower phases were combined, concentrated under reduced pressure at 40° C and dried in vacuum oven. The dried product obtained was washed with 10 ml of ether two times, dried and dissolved with 50 ml of glacial acetic acid. The solution was then quantitatively mixed with ferric chloride acetic acid reagent and heated over a water bath at 60° C for 25 min. Up on the formation of stable colour, the absorbance was measured at 409 nm. The concentration of saponin in the crude extracts was calculated using the calibration curve equation.

2.1.2.5.3 Preparation of extract for formulation

Various crude extracts of the seeds were prepared using 60%, 70% and 80% methanol as described above. The extracts were characterised in terms of their physico-chemical properties and suitable extract was formulated.

2.1.2.5.4 Determination of traditional dose

20 g of the defatted powdered seeds were macerated with 500 ml of 5% ethanol in water over night. After 24 hrs the extract was filtered and dried. The amount of saponins in the dry extract was determined colourimetrically as described above. Equivalent amounts of the crude extract containing the same amounts of saponins were taken as the dose.

2.1.2.6 Characterisation of the dry extract

2.1.2.6.1 Particle size analysis

Particle size distributions of the powdered extracts were measured using a Sympatic laser diffraction spectrometer (Sympatec GmbH, Clausthal-Zellerfeld, Germany). A 200 mm focal length lens, a pressure of 4 bar and a measuring time of 5 second were used.

2.1.2.6.2 Morphology

Surface structures of the powdered extracts obtained by vacuum oven and freeze drying, the n-butanol fraction and the purified powders were determined using a scanning electron microscope. Particles were sputtered with gold and scanning electron micrographs were taken with a DSM 940 apparatus (Carl Zeiss, Oberkochen, Germany). The accelerating voltage and the magnification are shown on the photographs.

2.1.2.6.3 Water uptake

The water sorption pattern of the crude, n-butanol fraction and the purified extracts were determined using computerised analyser (Krüss, processor Tensiometer K12,

version 4.04). Different relative humidities 33%, 44%, 59%, 65%, 75% and 85% were prepared using saturated salt solutions of potassium chloride, sodium chloride, sodium nitrite, sodium bromide ($NaBr.2H_2O$), potassium carbonate (K_2CO_3 $1.5H_2O$) and magnesium chloride ($MgCl_2.6H_2O$) respectively. Powder bed of 1 gram of the extract was prepared on the balance hanged in the humidity chamber. The weight gain per unit time was recorded by the processor. All the extracts were kept in desicator over silica gel for three days before conducting the experiments.

2.1.2.6.4 Water content determination

The moisture content of the various crude extracts was measured using computerised Karl Fischer water-titration set-up (Karl Fischer, Deutsche Metrohm, GmbH & Co., Elektronische Messgeräte, Filderstadt, Germany). In this, 35 mg of the crude extracts of the seeds of *G. lotoides* were dissolved in methanol: formamide (2:1) and titrated with Hydranal® composite 2, (one component reagent for volumetric Karl Fischer titration). The amount of Hydranal® composite 2 consumed was noted and the water contents of the extracts were calculated using a programme called "702 SM Tritrion". Standard sodium tartarate dihydrate, which contains 15.66% water was used as standard.

2.1.2.6.5 Densities

Bulk density

100 g of the powdered extracts were poured into a 250 ml glass measuring cylinder and the volume was noted. The bulk densities were determined from known mass and bulk volume.

Tapped density

100 g of the powdered extracts were poured into a 250 ml glass measuring cylinder, tapped 1250 times using an Engelsmann tap meter (JEL ST 2, Engelsmann AG, Ludwigshafen, Germany) and the volume was noted. The tapped densities were determined from known mass and tapped volume.

True density

Air comparison pycnometer (Beckman GmbH, 8000 Munich, Germany) was used to determine the true densities of the powdered extracts. Atmospheric air was used as a comparison gas.

2.1.2.6.6 Powder flow properties

Angle of Repose

Angle of repose was determined by pouring 150 ml of the powdered extract through a funnel of 10 mm pore size, adjusted at 75 mm height from the base, into 10 cm diameter plate placed below the tip of the funnel.

Flow rate

The flow rate of the powdered extract was determined by pouring 100 gram of the powdered extracts through a funnel of pore size 10 mm with closing end. The amount of extract passing per unit of time under gravitational force was recorded.

2.1.2.6.7 Compactability of the extracts

Mixtures of the crude extract and "lubricant A" were compressed at various compression forces using an instrumented eccentric tablet machine (Korsch EK 0, E. Korsch GmbH, Berlin, Germany) fitted with 12 mm flat-faced punches.

2.1.2.7 Preparation of tablets

2.1.2.7.1 Tablet formulations

Eight formulations with four different types of filler/binders (coded as filler/binder A, B, C and D) and two disintegrants (coded as disintegrant A and B) were prepared. Formulations were compressed into 1.15 g oblong tablets and batch sizes of 34.5 g were used.

All the powders except the lubricant were sieved with 0.8 mm sieve (0.315 mm for the lubricant) before mixing. Appropriate amounts of the extract and the fillers/binder were mixed in a Turbula mixer (Turbula T2C, W. Bachofer AG, Basel, Switzerland) for 5 min at 42 rpm. To the mixture the specified amounts of disintegrant and glidant were added and mixed in the Turbula mixer for another 5 min. The lubricant was then added and the mixture was mixed in the Turbula mixer for further 5 min.

2.1.2.7.2 Compression of tablets

Formulation mixtures were compressed at approximately 15 kN on an instrumented eccentric tablet machine (Korsch EK 0; E. Korsch GmbH, Berlin, Germany) fitted with oblong punches and the effect of types of filler/binders and disintegrants were evaluated.

In order to investigate the effect of compression force, formulation containing filler/binder A and disintegrant A was prepared and compressed at approximately 6.5, 8.4, 12.1, 15.5, 18.4, and 22.8 kN. The lower punch holder was instrumented with strain gauges (3/120 Ly11, Hottinger Baldwin Meβtechnik, Darmstadt, Germany). Signal transfer was achieved by a carrier frequency bridge (PR 9307, Philips GmbH,

Kassel, Germany). Compression data were collected and analysed using a programme called "Messfix".

2.1.2.8 Evaluation of tablet properties

2.1.2.8.1 Crushing strength

The crushing strengths of the tablets were measured 24 hrs after compression using Schleuninger tester (Model 6D; Dr. K. Schleuninger, Zürich, Switzerland). 10 tablets from each batch were determined and the average value was taken.

2.1.2.8.2 Friability

Roche friability tester (Erweka GmbH, Germany) was used to measure the friability of the tablets. In this, 10 tablets from each batch were weighed and run for 5 min. On completion, the dust was removed and the percent loss in weight of the tablets was calculated as the friability.

2.1.2.6.3 Disintegration time

The disintegration times of the tablets were measured using disintegration apparatus (Pharma Test, Type PTZ 1, Hamburg, Germany), in 700 ml demineralised water at 37° C. Tablets were considered completely disintegrated when all particles passed through the wire sieve.

3. RESULTS AND DISCUSSION

3.1 Extraction and purification of saponins from the seeds of *G. lotoides*

Scheme 3.1 shows the extraction and purification of saponins from the seeds of *G. lotoides*. Quantitative extraction of the seeds with 80% methanol in water using ultura-turrax mixer at 9500 rpm for 10 min yielded 11.2% crude extract, 3.2% n-butanol fraction and 2.4% purified extract (total saponins) calculated on the basis of dry defatted seeds.

Previous extraction works conducted on the plant have utilised 80% methanol as extracting solvent (54-56) and reported 10% yield of crude extract (56). In this experiment, relatively more yield was obtained using vortical extraction. In vortical or turbo-extraction, the drug to be extracted from the plant material is stirred in the mensutrum with a high-speed mixer or homogeniser, i.e., ultura-turrax. The shredding and shearing forces break down the drug material to a particle size, which is much smaller than that of the starting material. The cells become highly disintegrated and diffusion of the drug substances through the cell membrane is largely replaced by washing out from the destroyed cellular tissues. This treatment results in substantially faster extraction and better yield (30).

Total extraction of saponins from plant materials is usually difficult and sometimes impossible. This challenge is usually associated with their relatively large molecular weights and high polarity (71). As mentioned above, various extraction methods yield different amounts of saponins. Moreover, any alteration in the extraction processes such as, solvent ratio, rate of mixing, time of extraction, etc., may alter both the quantitative ratio and the qualitative composition of the extract (30). Hence, prior to

Powdered seeds

↓ Defatted with 50 ml hexane 3 times, (for each 5 g)
Stirred with ultura-turrax 9500 rpm for 10 min,
Filtered and dried.

Defatted powder (5 g)

↓ Extracted with 50 ml of 80% methanol 3 times;
Stirred with ultura-turrax 9500 rpm for 10 min;
Filtered and filtrate combined.

80% methanol extract

↓ Concentrated on rota vapour at 40° C
Dried (freeze dried or vacuum oven dried)

Dry crude extract (0.56 g, 11.2%)

↓ Dissolved in 30 ml of water
Partitioned between n-butanol (1:1)

n-Butanol layer | Aqueous layer

↓ Concentrated in rota vapour (40° C);
Dried in desciator (silica gel)

Dry Extract (n-butanol fraction) (0.16 g, 3.2%)

↓ Dissolved in 2 ml methanol
Added into 40 ml of diethyl ether

Precipitate (total saponins)

↓ Centrifugation (2000 rpm for 20 min, at 5 °C)
Dried in desicator

Dry product (purified extract) (0.12 g, 2.4%)

Scheme 3.1: Extraction and purification of saponins from the seeds of *G. lotoides*

development of standards and before proceeding with further extraction work, every step of the extraction has to be standardised and validated.

3.1.1 Standardisation of the extraction method

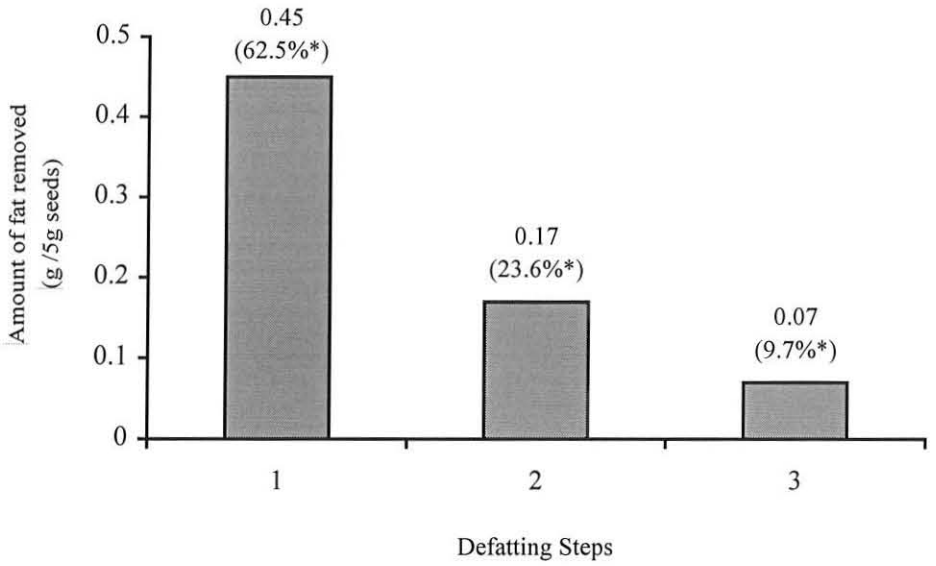
3.1.2 Defatting and determination of the total fat content

Removal of fats from plant materials, particularly from seeds and other high fat-containing organ, is advisable prior to the main extraction step. Presence of fats in the starting materials not only interferes with the extraction and drying process but also with formulations. Defatting process prior to extraction of seeds of *G. lotoides* have been reported (54-56).

5 g of the powdered seeds of *G. lotoides* were defatted three times successively with 50 ml of hexane using ultura-turrax mixer at 9500 rpm for 10 minutes. The amount of fat removed at each defatting steps were determined and compared to the total fat content of the seeds. Total fat content of the seeds of *G. lotoides* was determined by defatting with large volume of hexane (i.e., 5 g of the seeds with 100ml n-hexane, five times) repeatedly until the n-hexane extract becomes colourless. After complete removal of hexane, the total content of the fat was found to be 14.3 % (0.715 g from 5 g seeds). The results obtained are in good agreement with previous work, which reported a total of 14% oil (64).

As shown in Figure 3.1 the amount of fat removed during the first defatting step is only 62.5% of the total fat. The second and the third defatting steps removed 23.6% and 9.7% respectively. The three successive defatting procedures removed a total of 95.8%

of the total fat. Hence, the results obtained indicate that, all the three defatting steps are important to remove most of the fat.



*percent removed out of the total fat content

Figure 3.1: Amount of fat removed from 5 g of powdered seeds of *G. lotoides* in three successive extraction steps.

3.1.3 Determination of total saponin contents of the seeds of *G. lotoides*

Total extraction of saponins form plant material is usually difficult (71). Knowing the total amount of saponin in the plant material, however, is important for the development of analysis as well as to select suitable solvent system for a maximum yield of saponins. Hence, 5 g of the defatted seeds were extracted ten times repeatedly each with 100 ml of 80% methanol, (i.e., a total of 1 litre) until the final filtrate appeared colourless. The filtered extracts were combined, concentrated under reduced pressure at 40° C and dried. The amount of the crude extract, n-butanol fraction and the purified extract were determined following the flow chart in Scheme 3.1.

The results indicate that 0.9 g (18%) of crude extract, 0.34 g (6.8%) of n-butanol fraction and 0.18 g (3.6%) of purified extract (total saponins) could be obtained from 5 g of defatted seeds. Hence, the extraction method described earlier removes only 67% of the total saponins present in the seeds. (i. e., only 0.12 g of saponins was extracted from the total 0.18 g). The observed result indicates the challenge posed by saponins in their total extraction from plant materials.

3.1.4 Determination of suitable solvent system for a maximum yield

The type and relative amounts of saponins extracted could be highly varied depending on the proportion of water used for the extraction (73,74). Determination of solvent system that gives a maximum yield has also economical importance. It is, therefore, obvious that the correct choice of extraction procedure and solvent system is an extremely important and first foremost step.

Although numerous variations are possible, the most efficient extraction of crude saponins from dry plant material is achieved with aqueous methanol. Water alone is a less efficient extraction solvent for saponins, unless specifically water-soluble glycosides are desired. However, it has the advantage of being easily lyophilised and yields a cleaner extract (71).

As indicated earlier, previous extraction works conducted on the seeds of *G. lotoides* have utilised 80% methanol in water. However, there is no evidence or any attempt conducted to show the suitability of this solvent system over others with respect to its saponin yield. Hence, the extracting power of various proportions of aqueous methanol solvents has been tested for a maximum yield of saponin in the crude extracts.

Table 3.1 shows the amount of the various extracts obtained using various proportions of solvent systems (0–100 % methanol in water). The amount of the crude extract, the n-butanol fraction and the purified extract (total saponins) were determined as described in Scheme 3.1. As shown in Table 3.1, the amount of the crude extract obtained increases with increasing proportion of water. The maximum being 16.5% with pure water. However, the n-butanol fraction and the purified extract showed maximum yield at 20% and 60% methanol respectively. This gravimetric method of analysis suggests that maximum yield of saponins (i.e., purified extract) could be obtained using 60% methanol as extracting solvent.

Table 3.1: Yield in percent of the extracts from 5 g of seeds of *G. lotoides* using various concentrations of methanol in water as extracting solvent.

Methanol in water (%)	Crude extract gram (%)	n-Butanol fraction gram (%)	Purified extract gram (%)
0	0.824 (16.5)	0.222 (4.44)	0.097 (1.94)
10	0.801 (16.0)	0.243 (4.85)	0.101 (2.01)
20	0.775 (15.5)	0.268 (5.36)	0.114 (2.28)
30	0.765 (15.3)	0.265 (5.30)	0.125 (2.49)
40	0.750 (15.0)	0.232 (4.64)	0.132 (2.63)
50	0.702 (14.0)	0.220 (4.40)	0.148 (2.95)
60	0.670 (13.4)	0.211 (4.22)	0.155 (3.10)
70	0.605 (12.1)	0.210 (4.20)	0.130 (2.60)
80	0.560 (11.2)	0.160 (3.20)	0.120 (2.40)
90	0.455 (9.1)	0.190 (3.80)	0.115 (2.30)
100	0.440 (8.8)	0.266 (5.32)	0.087 (1.73)

Formation of stable foam creates filtration problem in solvent systems containing 50% methanol and below during ultura-turrax extraction. Required amount of silicon dioxide anti-foam emulsion was used to solve this problem. Moreover, these solvents formed a

stable phase during liquid-liquid extraction with n-butanol, which is separated only after centrifugation at 2500 rpm for 4 min.

Percent total saponin content of the various crude extracts is shown in Figure 3.2. The percent total saponin contents of the crude extracts were calculated based on the purified extract (total saponin) yields determined using gravimetric analysis. As shown in the figure, higher yield of total saponin were obtained with solvent systems containing high proportion of methanol (i.e., greater than 50% MeOH in water). Hence, 23.1% of the 60% methanol crude extract is composed of the total saponins, whereas, pure water (i.e., 0% methanol) crude extract contains 11.7% of saponin. Moreover, calculation of the amount of saponins extracted out of the total saponin contents, revealed that, 60% methanol extracted 86% of the total saponins present in the seeds of *G. lotoides* (i.e., 0.155 g from the total 0.18 g of saponins).

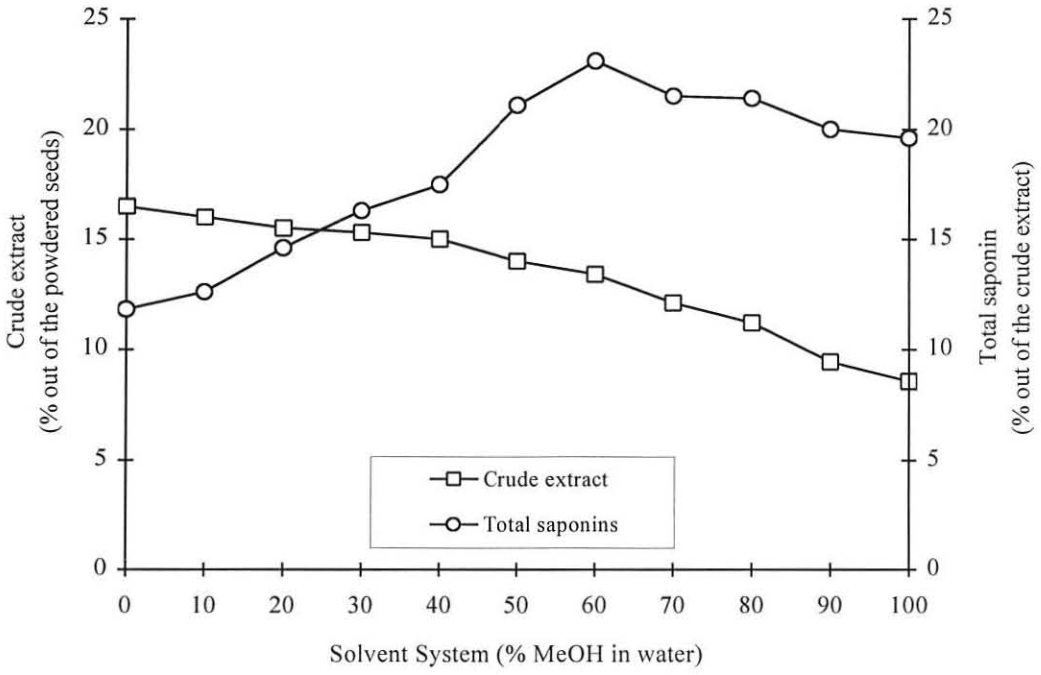


Figure 3.2: Percent yield of the crude extract and their percent saponin contents using various proportions of water/methanol as extracting solvents.

3.2 Assay development

The first step in preformulation or formulation studies is to develop an analytical method so that all future measurements can be quantified. Various methods have been employed for the qualitative and quantitative determinations of saponins in crude extracts and their phytopharmaceutical preparations. Determination of saponins based on their classical properties, such as: haemolysis, surface activity, piscicidal activity (fish toxicity) and foam-formation, have largely been replaced by photometric methods such as colourimetry of derivatives, TLC-densitometry, GC and HPLC (68,69). In this work, attempts have been made to develop quantitative methods, namely; colourimetric, UV-spectrophotometric, and TLC-densitometric for measurement of saponins in the seeds of *G. lotoides*

3.2.1 Colourimetric method

The colourimetric method of determination of saponins in the extract of the seeds of *Aesculus hippocastanum* L. (horsechest nut) which is official in German Pharmacopoeia was adopted with minor modification (72). ß-escin, which is a mixture of triterpenoidic saponins from *A. hippocastanum*, was used as a reference standard to measure the saponins in the purified extract of the seeds of *G. lotoides* as ß-escin-equivalent.

In the present method, saponins were reacted with colour-forming agent, ferric chloride in acetic acid/sulphuric acid, and the absorbance of the coloured-solution formed was measured at the appropriate wavelength. The reaction between saponins and ferric chloride was reported to be specific and gives red colour with different absorption maximum for saponins from different sources. Moreover, the method is suitable for

quantitative analysis, as the intensity of the red colour formed is directly proportional to the amount of saponins present in it (71).

As shown in figure 3.3 and 3.4, the absorption spectra of saponins of *G. lotoides* and ß-escin-ferric chloride complex showed maximum at 409 and 460 nm respectively. On the other hand, German Pharmacopoeia (DAB) recommends a wavelength of 540 nm for colourimetric determination of ß-escin in the extracts of *A. hippocastanum*.

Colourimetric method of determination saponins has been reported to be sensitive and selective. However, the procedure requires pre-separation steps of the crude extract to remove flavonoids, sugars and phytosterols, which can react with ferric chloride and produce colour (71). Hence, the crude extract was purified prior to reacting with the colour-forming reagent. In this, 50 mg of the extracts of *G. lotoides* was dissolved in 60 ml of 0.1 N HCl. Ultrasonic shaking was employed to facilitate the dissolution and the dissolved parts were filtered. The filtrate was then mixed with 40 ml of 1-propanol and 100 ml of chloroform in separatory funnel. The mixture was thoroughly mixed for 2 min, allowed to separate and the lower phase was taken and dried. The dried product was then washed with ether and dried.

Saponins of *G. lotoides* were found to be less soluble in water and when the acidic solution was mixed with 1-propanol and chloroform, the saponins will accumulate in the chloroform saturated phase (i.e., lower phase), whereas sugars and other highly polar compounds will remain in the aqueous phase. Phytosterols also partitioned together with saponins into the chloroform layer and they are removed during washing with ether.

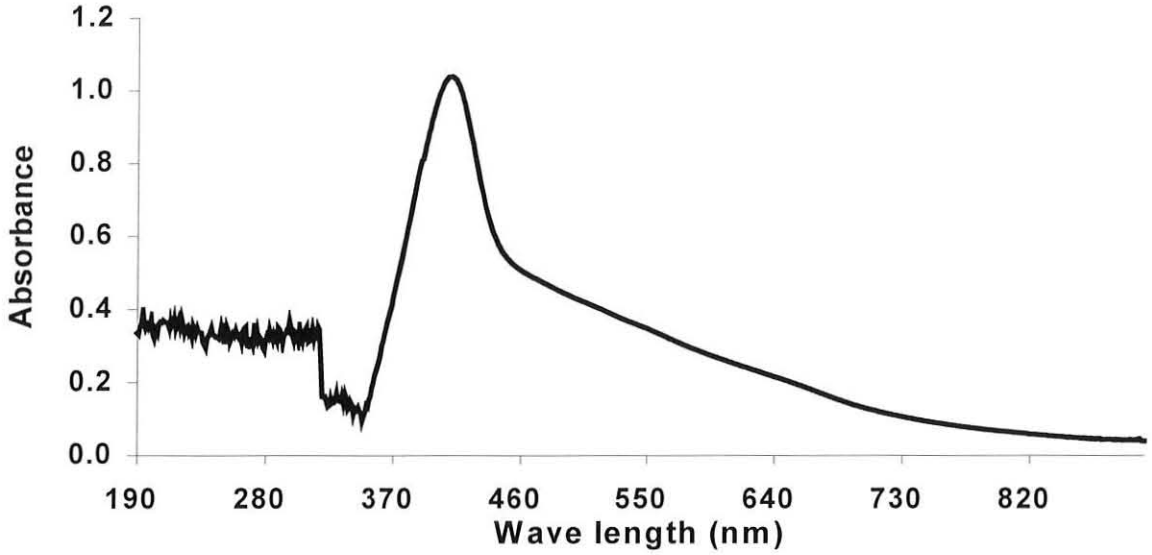


Figure 3.3: Absorption spectrum of saponins of *G. lotoides*-ferric chloride complex (0.2 mg/ml).

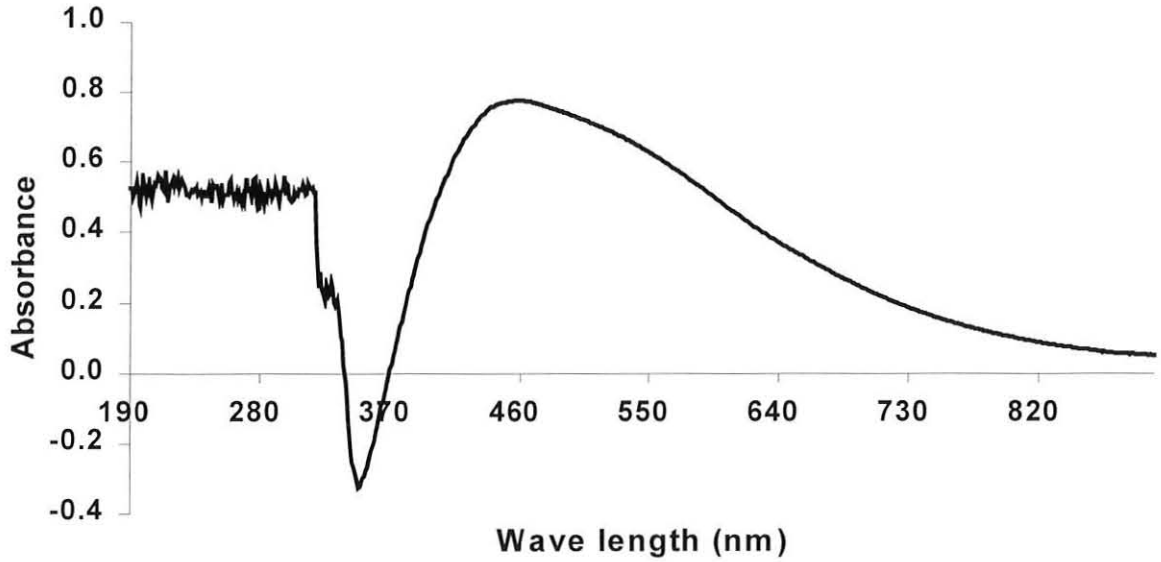


Figure 3.4: Absorption spectrum of β-escin-ferric chloride complex (0.2 mg/ml).

The final dried purified extract was then dissolved in glacial acetic acid and reacted with ferric chloride-acetic acid reagent. The test solutions, standard solutions as well as the colour-forming reagent (i.e., ferric chloride-acetic acid) were prepared fresh and used immediately (75).

Quantitative analysis work requires standard or calibration curve against which all measurements are conducted. Hence, solutions having known concentrations of the standards (i.e., saponins of *G. lotoides* and β-escin-ferric chloride complex) were prepared and calibration curves were made at 409 and 540 nm for saponins of *G. lotoides* and at 460 and 540 for β-escin. (Figures 3.5, 3.6, 3.7 and 3.8).

Figure 3.5 shows the calibration curve and the residual plots of β-escin in concentrations ranging form 0.02 – 0.12 mg/ml at 540 nm. Linear regression of the standard curve yielded an equation $Y = 6.6314X - 0.0279$ (where Y is the absorbance and X is concentration) with a correlation coefficient of 0.9983. The simple and ten times confidence interval and the residual plots showed a uniform distribution and were found to be within acceptable range.

On the other hand, as shown in Figure 3.6, the calibration curve of saponins of *G. lotoides* at 540 nm showed wider confidence intervals. Linear regression of the standard curve over a concentration range of 0.02 – 0.12 mg/ml yielded an equation $Y = 3.168X - 0.0235$ with correlation coefficient of 0.9954. Moreover, the analysis saponins of *G. lotoides* showed less sensitivity at this wavelength as the absorbance of 0.12 mg/ml solution was only 0.364.

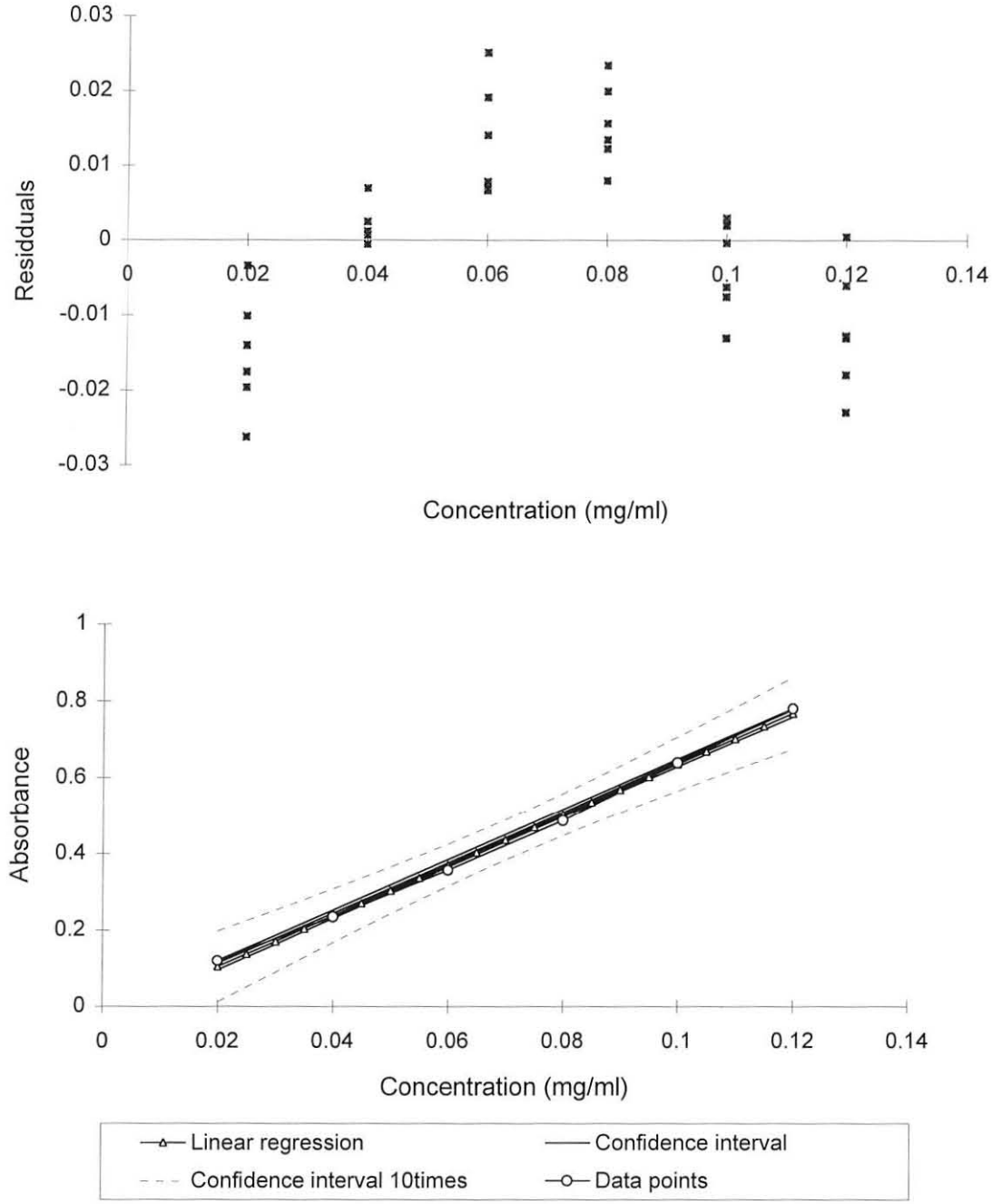


Figure 3.5: Calibration curve and residual plots of β-escin-ferric chloride complex at 540 nm.

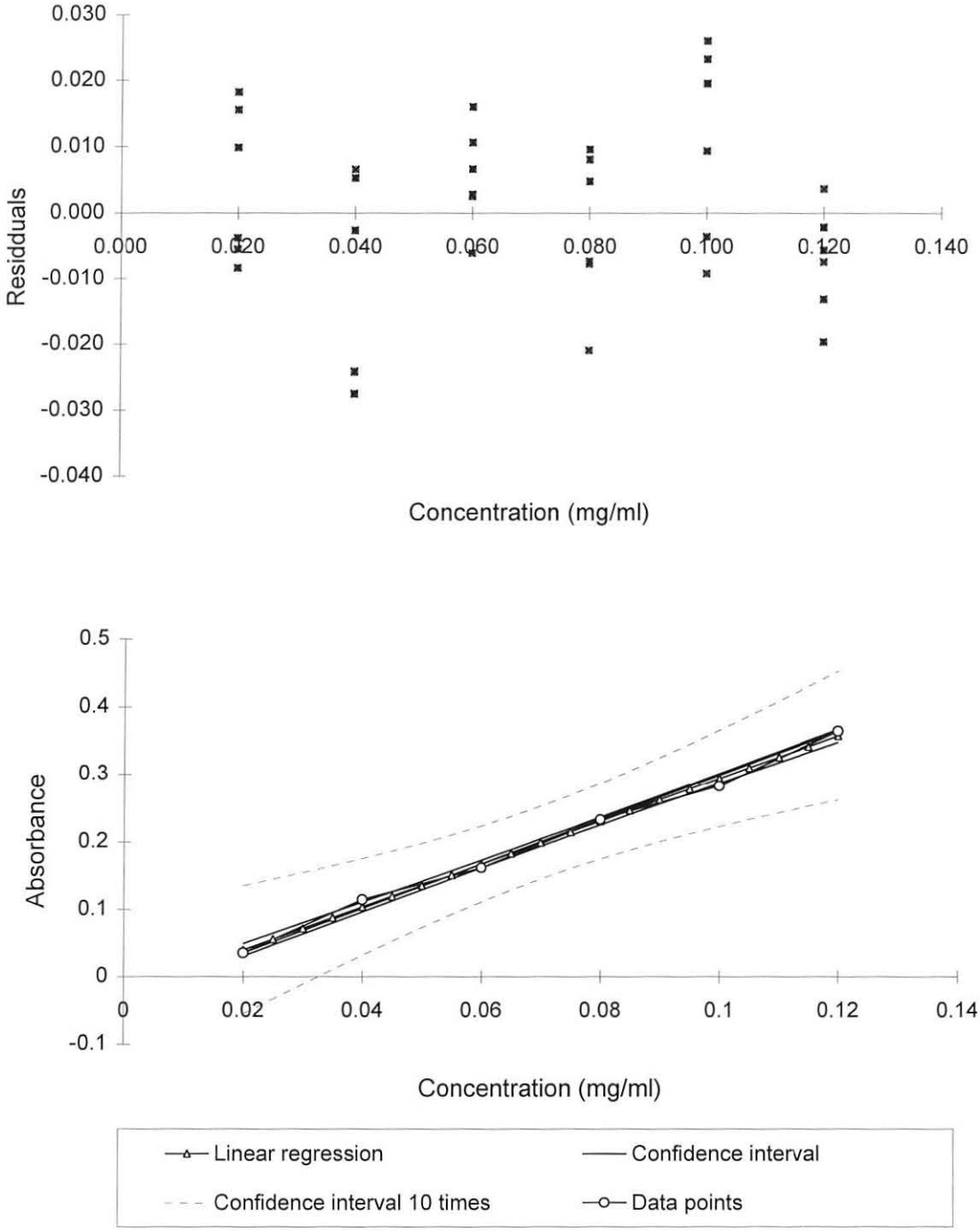


Figure 3.6: Calibration curve and residual plots of saponins of *G. lotoides*-ferric chloride complex at 540 nm.

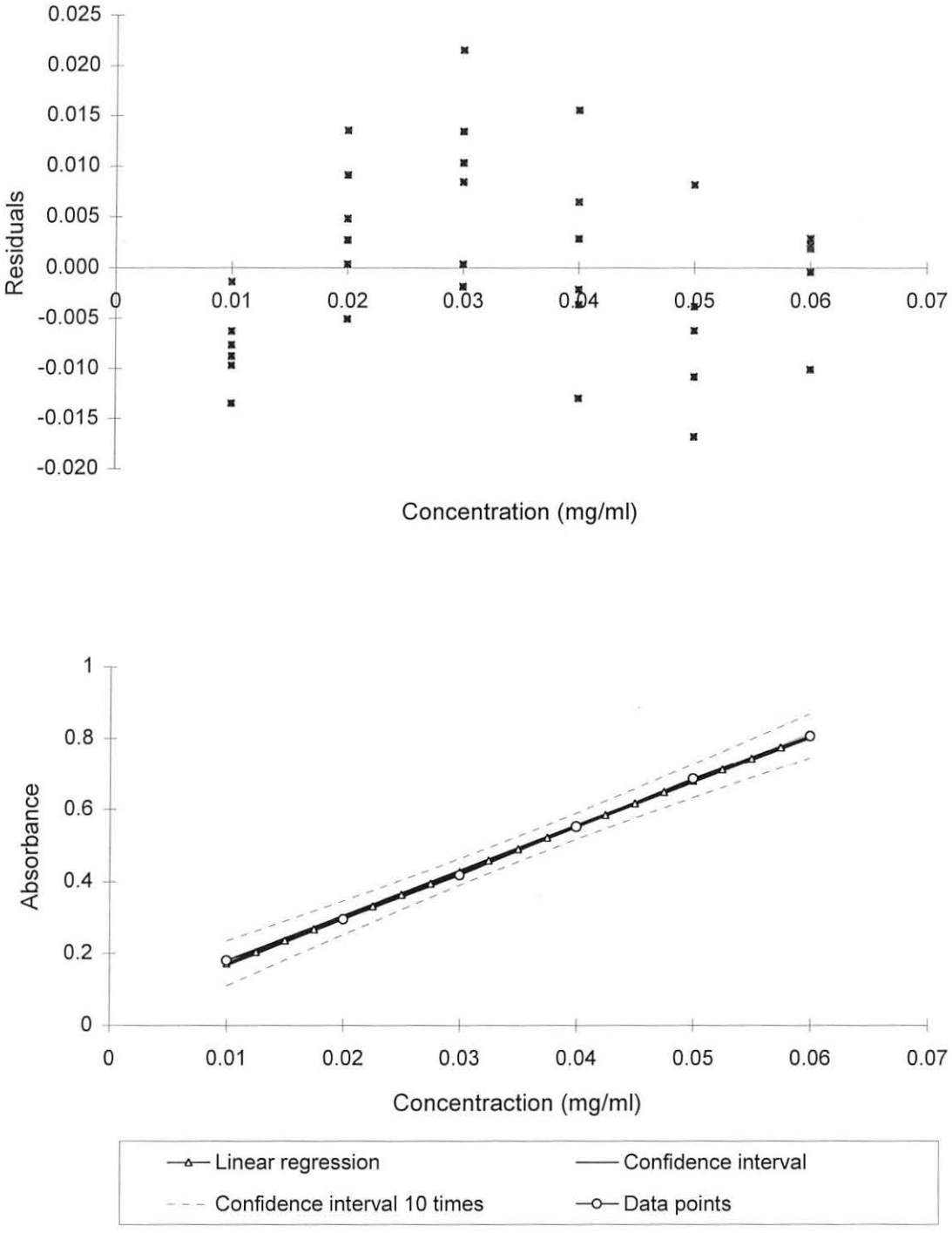


Figure 3.7: Calibration curve and residual plots of saponins of *G. lotoides*-ferric chloride complex at 409 nm.

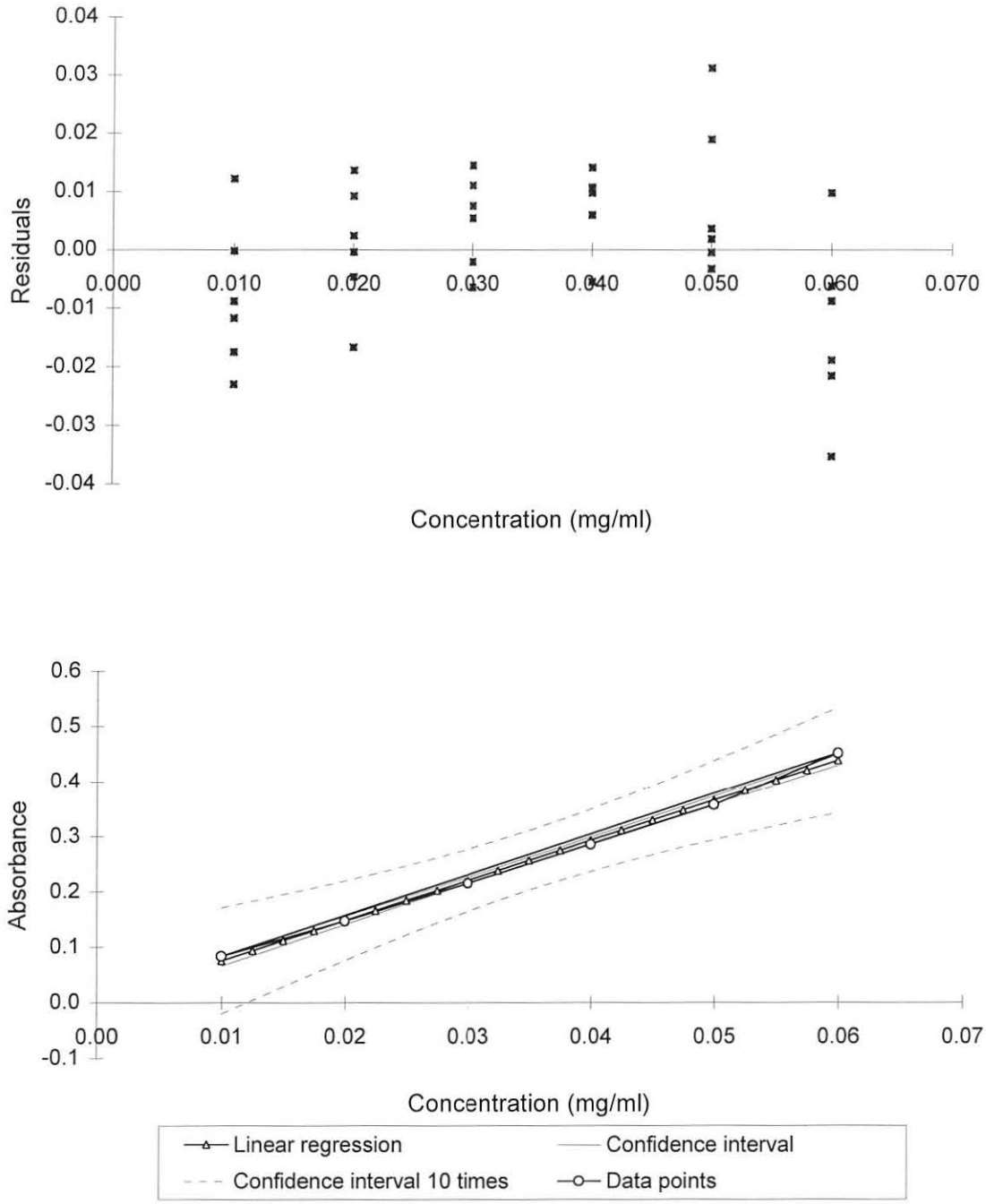


Figure 3.8: Calibration curve and residual plots of β-escin-ferric chloride complex at 460 nm.

The problems of sensitivity and accuracy at 540 nm (Fig. 3.6) were solved when the calibration wavelength of saponins of *G. lotoides* was done at the maximum absorbance at 409 nm (Fig. 3.7). The calibration curve and the residual plots of saponins of *G. lotoides* over concentration range of 0.01–0.06 mg/ml at 409 nm showed narrow confidence intervals with a well distributed residual plots. Linear regression of the standard curve yielded an equation Y = 12.669X + 0.0456 with correlation coefficient of 0.9993. Hence, the method was considered to be sensitive as the Beer's law was obeyed over a concentration range of 0.01–0.06 mg/ml.

Similarly, the calibration curve and the residual plots of β-escin were determined at 460 nm, i.e., its λ max (Fig. 3.8). However, as the confidence intervals and the correlation coefficient show, the sensitivity and accuracy at this wavelength is inferior to that of 540 nm. Linear regression of the standard curve yielded an equation Y = 7.244X + 0.0027 with correlation coefficient of 0.9942.

Based on calibration results (Figure 3.5–3.8), wavelengths of 409 and 540 nm were selected for standard curves of saponins of *G. lotoides* and β-escin respectively.

Validation of precision and accuracy are the most important criteria in determining whether a method is suited to a particular task and whether data generated under routine use of a analytical method are acceptable (acceptance criteria) (76).

Precision is often carried out by establishment of a criterion on relative standard deviation. This criterion is generally established based on the method and the requirements of the results and usually expressed as standard deviation. Analysis of the

calibration data of β-escin and saponins of *G. lotoides* showed standard deviations of 0.0658 and 0.088 mg/ml respectively, which are acceptable.

Effect of heating time

Effect of heating time on the formation of red-coloured complex of β-escin-ferric chloride has been reported (75) and in this study, similar result was obtained (Fig. 3.9). As shown in the Figure 3.9 and 3.10, the absorption of the coloured solutions increases with increase in heating time until it reaches maximum at 20 min for β-escin and 25 min for saponins of *G. lotoides*, which then remains constant. Hence, it is essential to monitor the heating time and measuring the absorbance after the red colour formation is complete.

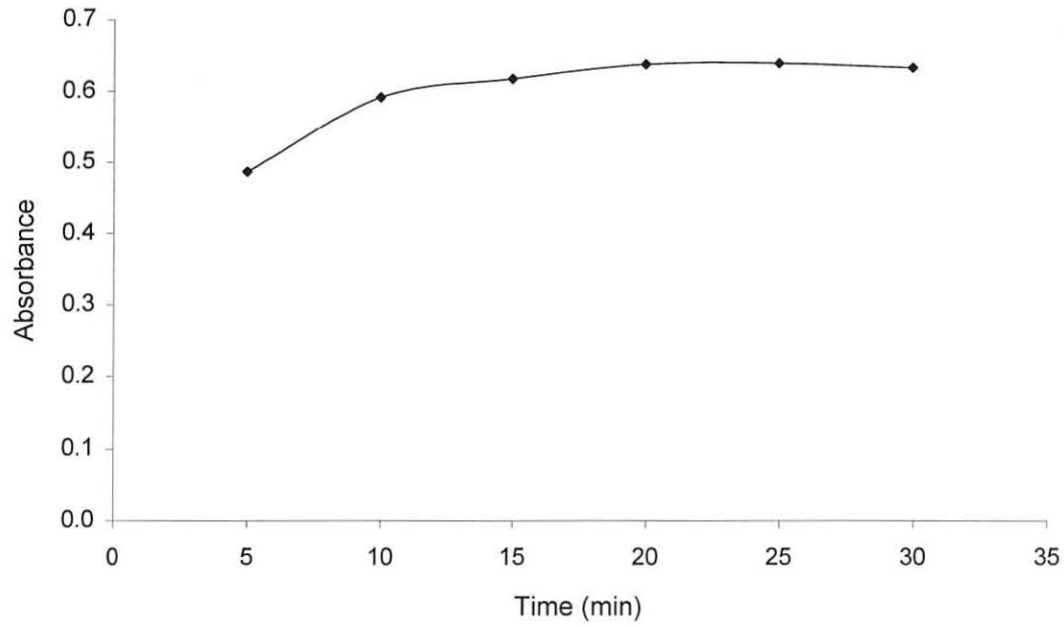


Figure 3.9: Effect of heating time on the absorbance profile of β-escin-$FeCl_3$ (0.01 mg β-escin /ml) at 540 nm.

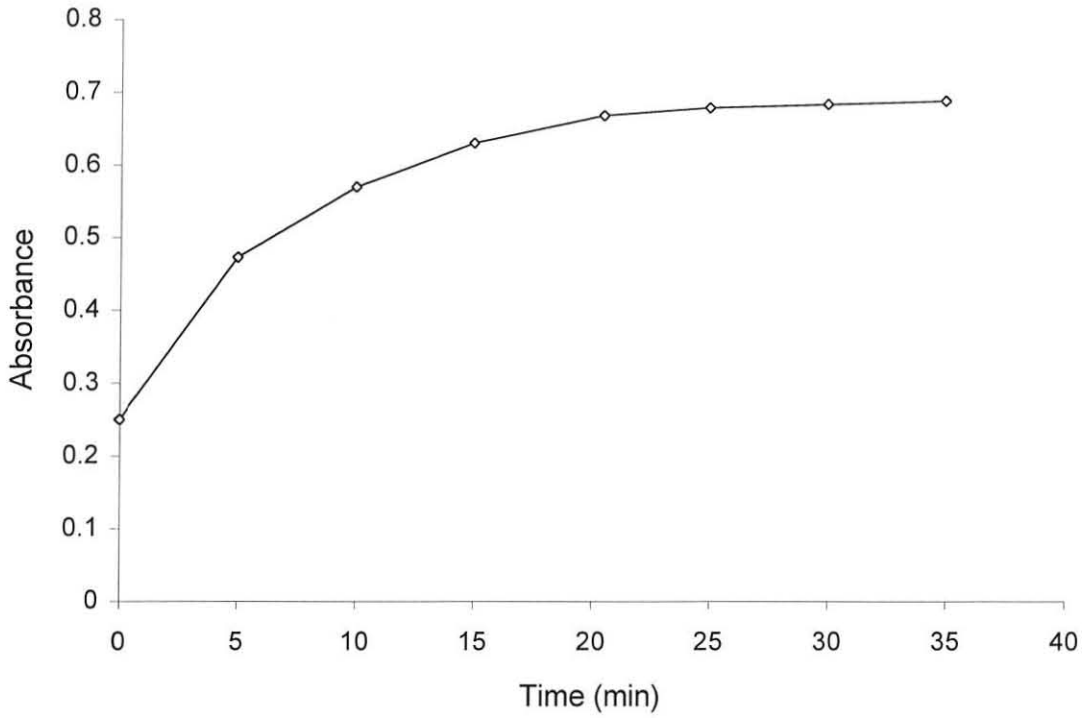


Figure 3.10: Effect of heating time on the absorbance profile of saponins of *G. lotoides*-$FeCl_3$ (0.05 mg saponins of *G. lotoides*/ml) at 409 nm.

Determination of saponin of *G. lotoides* in the crude extracts

Saponin contents of the various crude extracts of *G. lotoides* were determined using colourimetric analysis method developed at 409 nm. The saponin contents were calculated using an equation Y = 12.669X + 0.0456. Figure 3.11 shows percent yield of the various crude extracts and the total saponin content determined colourimetrically at 409 nm. As shown in the figure, 60% MeOH crude extracts contains 17% saponins whereas 80% MeOH contains 12.8% saponins. On the other hand, only 4.5% saponins were obtained in the crude extracts prepared with pure water as extracting solvent, which indicates water alone is not a good extraction solvent for saponins of *G. lotoides*.

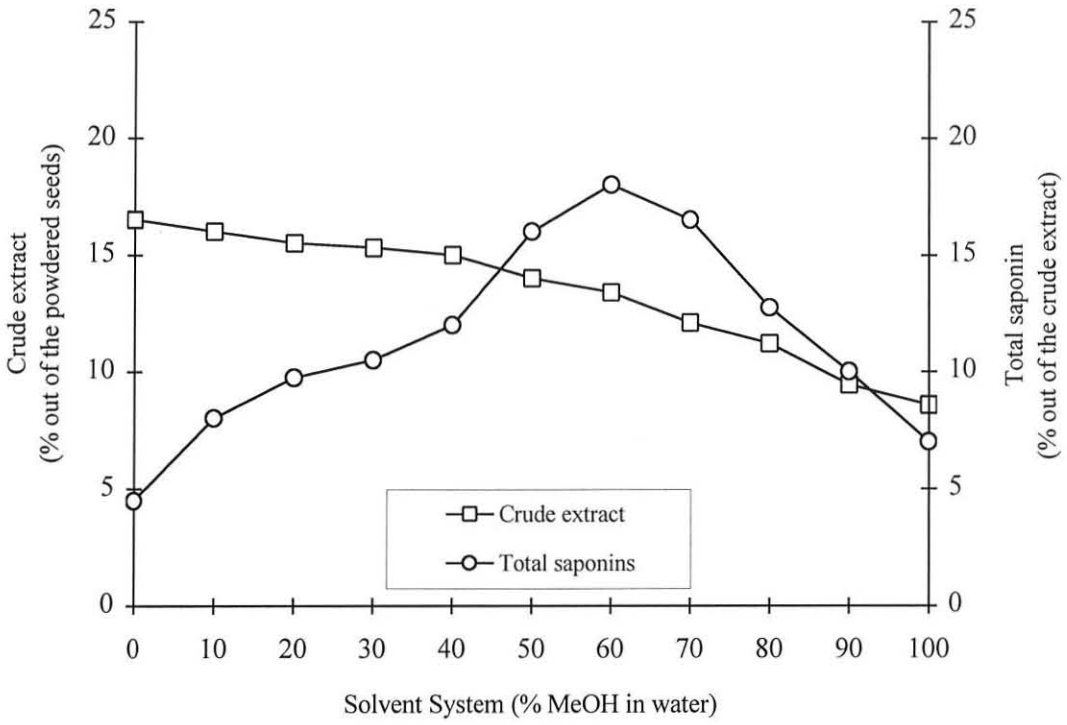


Figure 3.11: Percent yield of the crude extract and their percent saponin contents determined using colourimetric method at 409 nm.

3.2.2 UV-spectrophotometric method

Colourimetric methods are very sensitive but not suitable for estimating saponins in crude plant extracts without pre-separation of compounds that accompany the saponins, such as phytosterols and flavonoids (70).

In this work, quantitative UV-spectrophotometric analysis method for saponins of *G. lotoides* has been developed. The UV-spectrophotometric determination was carried out after derivatisation using 10% acetic acid in 0.1 N HCl. This method requires no pre-separation of the extracts to isolate accompanying compounds.

Figure 3.12 shows the absorption spectrum of the saponins of *G. lotoides* dissolved in 10% acetic acid in 0.1N HCl over a range of 220–290 nm. The absorption spectrum shows maxima at 238 and 269 nm. On the other hand, the absorption spectrum of ß-escin in 10% acetic acid in 0.1N HCl shows maximum at 238 nm (Fig. 3.13). The results obtained in this study are in good agreement with previous work conducted on spectrophotometric properties of β-escin (77).

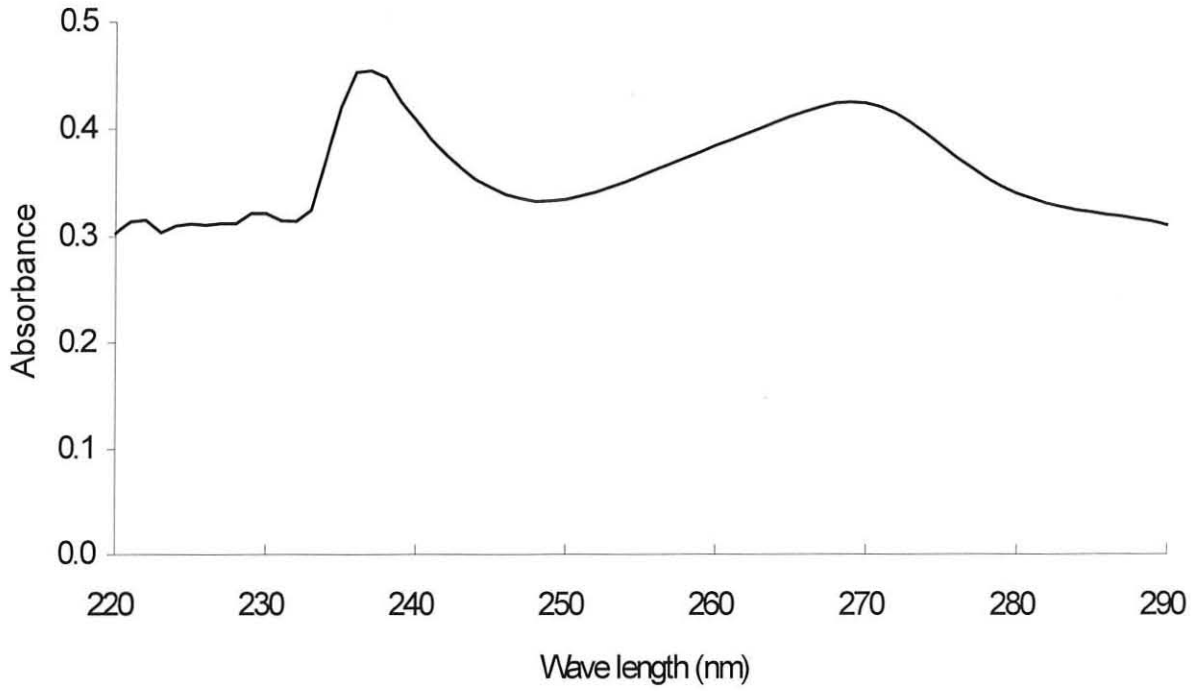


Figure 3.12: Absorption spectrum of the saponins of *G. lotoides* (0.06 mg/ml) in 10% v/v glacial acetic acid in 0.1 N HCl.

Solutions of saponins of *G. lotoides* having known concentrations (0.02-0.12 mg/ml) in 10% acetic acid in 0.1N HCl were prepared and calibration curve was made at 238 nm. (Fig. 3.14). As shown in Figure 3.14, the residual plots of saponins of *G. lotoides* in 10% acetic acid in 0.1N HCl are well distributed and the confidence intervals show

acceptable ranges. Linear regression of the standard curve yielded an equation of Y = 7.9514X - 0.0536 (where Y is the absorbance and X is concentration) with a correlation coefficient of 0.9983.

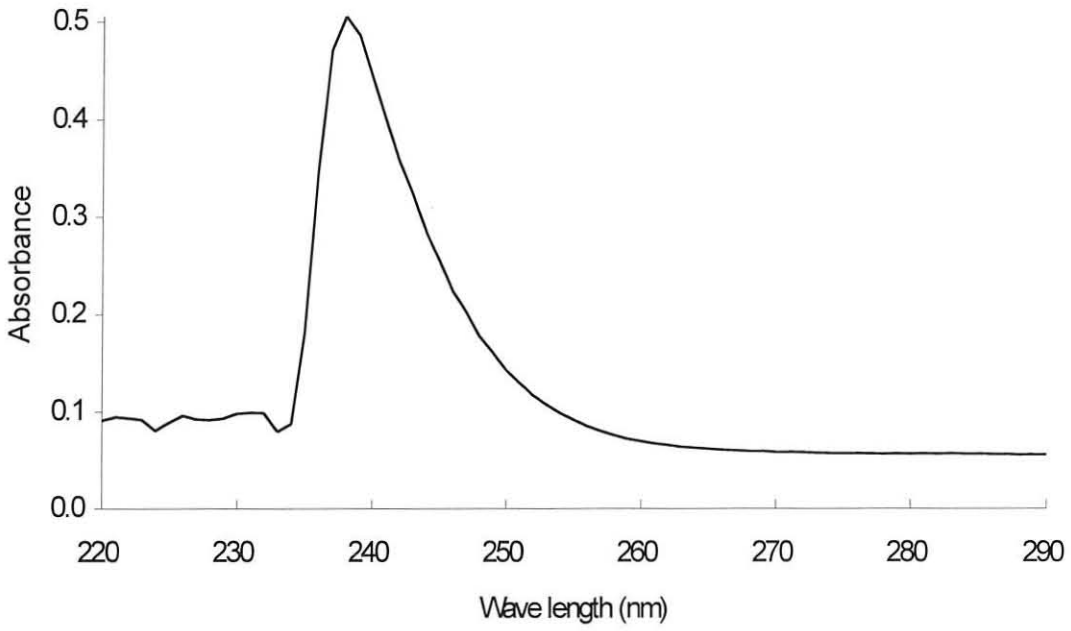


Figure 3.13: Absorption spectrum of β-escin (0.25mg/ml) in 10% v/v glacial acetic acid in 0.1 N HCl

The absorption spectrum of saponins of *G. lotoides* dissolved in glacial acetic acid (0.15 mg/ml) showed maxima at 271 and 332 nm (Fig. 3.15). 100% acetic acid was used as a background. The simplicity of the method and the typical absorption pattern could be utilised as a preliminary qualitative identification test. On the other hand, the absorption spectrum of ß-escin dissolved in 100% acetic acid showed only one maximum at a wavelength of 247 nm (Fig. 3.16).

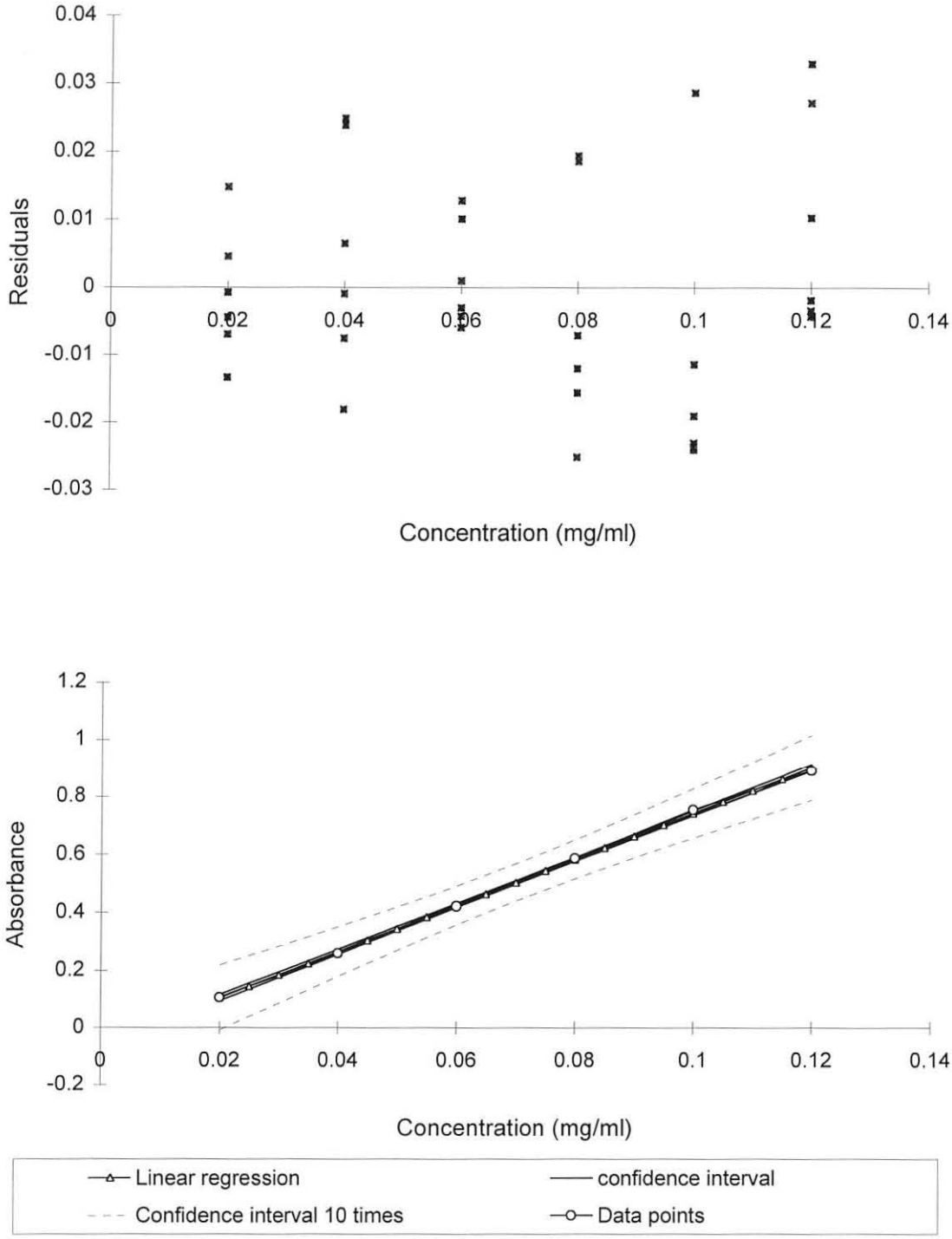


Figure 3.14: Calibration curve and residual plots of the saponins of *G. lotoides* dissolved in 10% acetic acid in 0.1 N HCl at 238 nm.

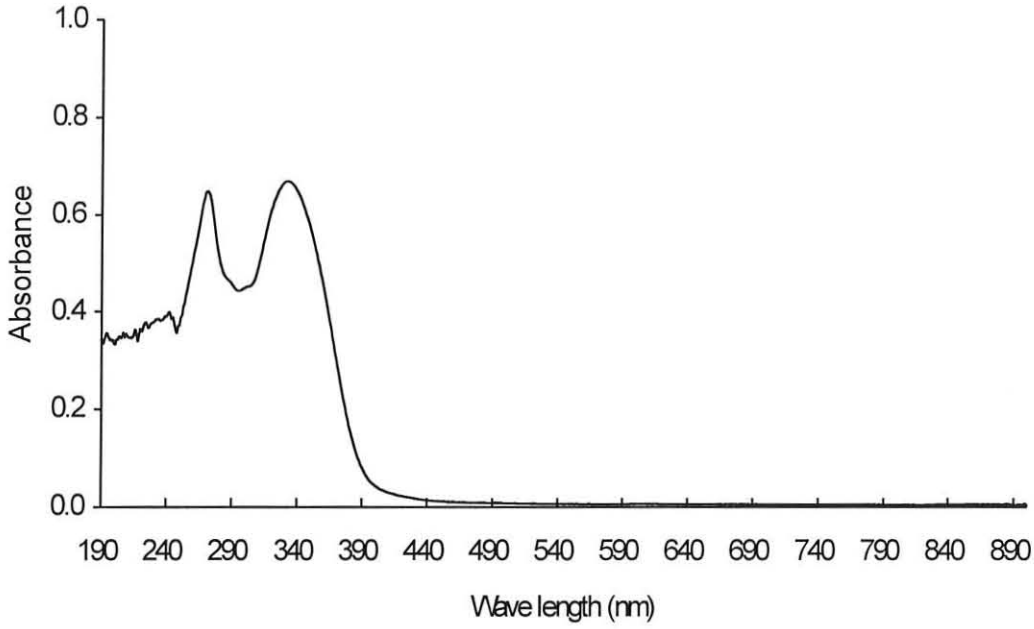


Figure 3.15: Absorption spectrum of the saponins of *G. lotoides* (0.15 mg/ml) in glacial acetic acid.

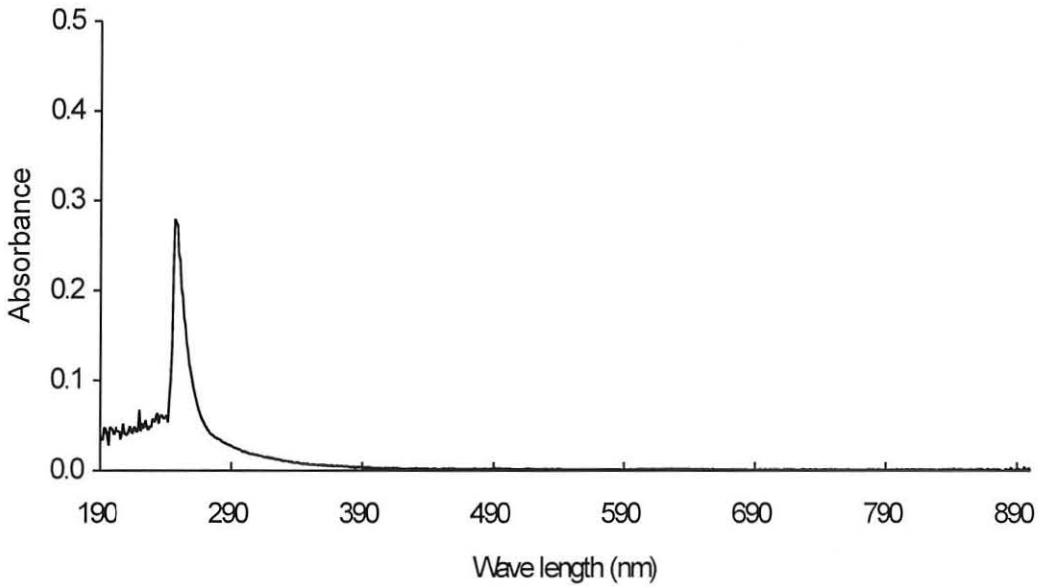


Figure 3.16: Absorption spectrum of β-escin (0.15 mg/ml) dissolved in glacial acetic acid.

3.2.3 TLC-densitometric method

TLC-densitometric method has been utilised in identification and quantitative determination of saponins such as β-escin in crude extracts and their phytopharmaceuticals. For instance, TLC-densitometric quantitative determination of β-escin in topical formulations such as, ointments has been reported (78) and the method was latter compared with HPLC quantitative analysis method and was found to be accurate, reproducible and in excellent agreement (79). TLC-densitometry was proposed for rapid quantitative determination of biologically active and/or characteristic constituents in plant materials and their phytopharmaceuticals (80).

TLC-densitometric quantitative determination method includes: a) preparation of test and standard solutions, b) application of equal volume of each solution to the plate, c) TLC development of the plates using suitable solvent system, d) solvent evaporation and development of spots using appropriate detecting reagent and e) measurement of spots density using suitable apparatus (78-80).

Attempts have been made to develop a TLC-densitometric quantitative determination method for saponins of *G. lotoides*. Quantitative measurements using TLC-densitometric requires appropriate TLC development with good resolution and separation of components. This includes selection of suitable solvent systems with reasonable base line separation of the components and appropriate detecting reagent. Hence, various types of solvent systems and detecting reagents were investigated.

Table 3.2 shows various types of mobile phases examined and the number of spots obtained. ß-escin was used as a reference standard. p-anisaldehyde reagent and

1% vanillin ethanolic solution were used as detecting reagents. As shown in Table 3.2, the seeds of *G. lotoides* contain up to 10 saponins.

Table 3.2: Number of the spots obtained using different types of mobile phases.

Types of Mobile phase	No. of spots (Saponins of *G. lotoides*)	No. of spots (ß-escin)
Acetic acid/water/n-butanol (10:40:50 v/v), Upper phase	7	3
Chloroform/methanol/acetic acid/ water (63:32:12:18 v/v), Lower phase	10	7
1,2 dichloroethane/ethanol/ methanol/water (50:20:20:6 v/v)	7	4
Chloroform/methanol/water/ Formic acid (50:40:8:1 v/v)	6	5

Other solvent systems used; namely, Ethyl acetate/methanol/Water (85:17:13 v/v), Ethyl acetate/2-butanone/water/ formic acid (50:30:20:10 v/v) and Toluene/ethyl acetate/Formic acid (40:10:5 v/v) showed poor separation of components.

Examples of chromatograms of β-escin and saponins of *G. lotoides* in different solvent systems are depicted in Figures 3.17-3.22. Figure 3.17 shows TLC-densitometry chromatogram of β-escin developed using upper phase of acetic acid/water/1-butanol (10:40:50 v/v) as a mobile phase. This method is official in German Pharmacopoeia for purity test of β-escin (72).

As shown in TLC-densitometry chromatogram of saponins of *G. lotoides* (Fig. 3.18, 3.20-3.22), base line separation of the peaks were not achieved with all the solvent systems examined. Hence, quantitative measurement of the saponins of *G. lotoides* was not successful with the solvent systems mentioned above.

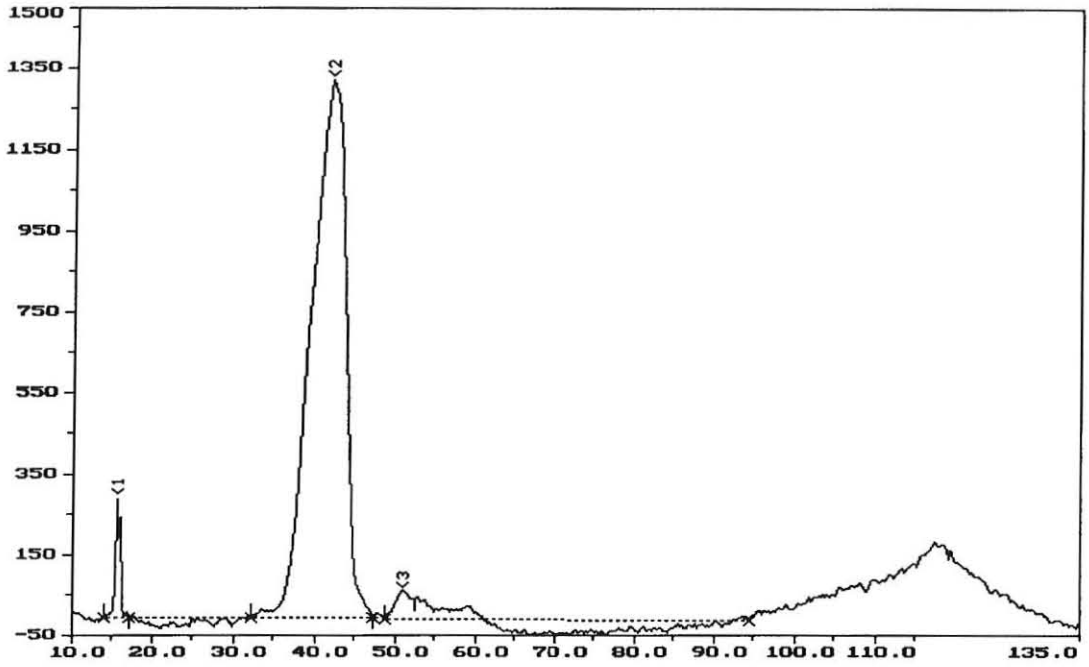


Figure 3.17: TLC-densitometry chromatogram of β-escin (mobile phase: upper phase of acetic acid/water/1-butanol (10:40:50 v/v)).

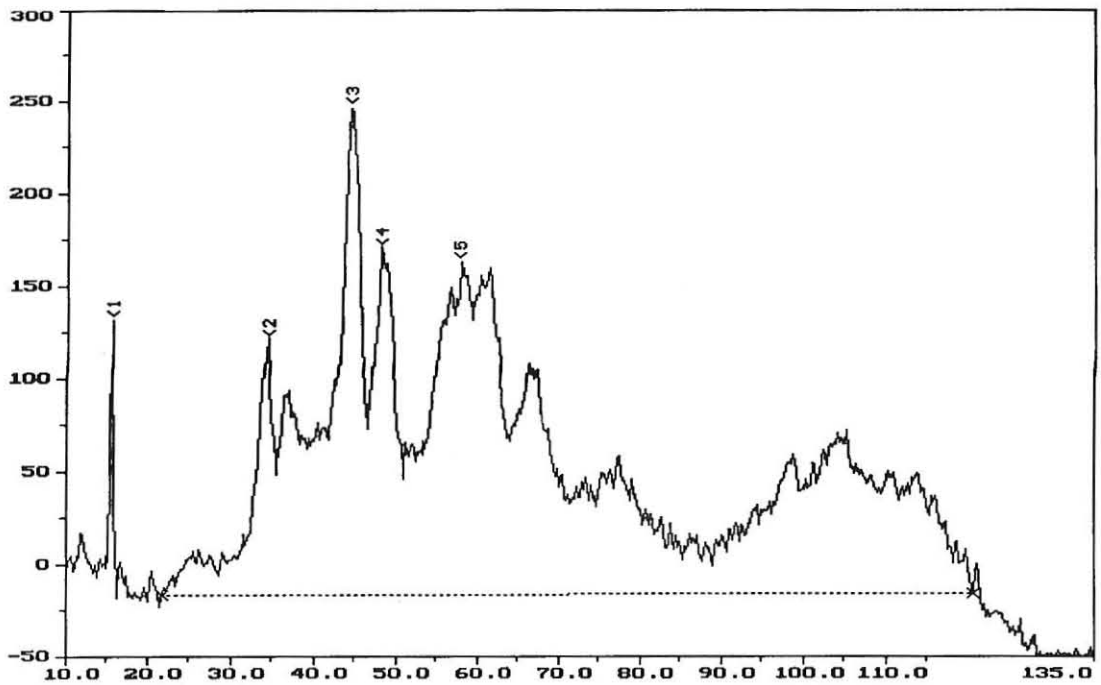


Figure 3.18: TLC-densitometry chromatogram of saponins of *G. lotoides* (mobile phase: upper phase of acetic acid/water/1-butanol (10:40:50 v/v)).

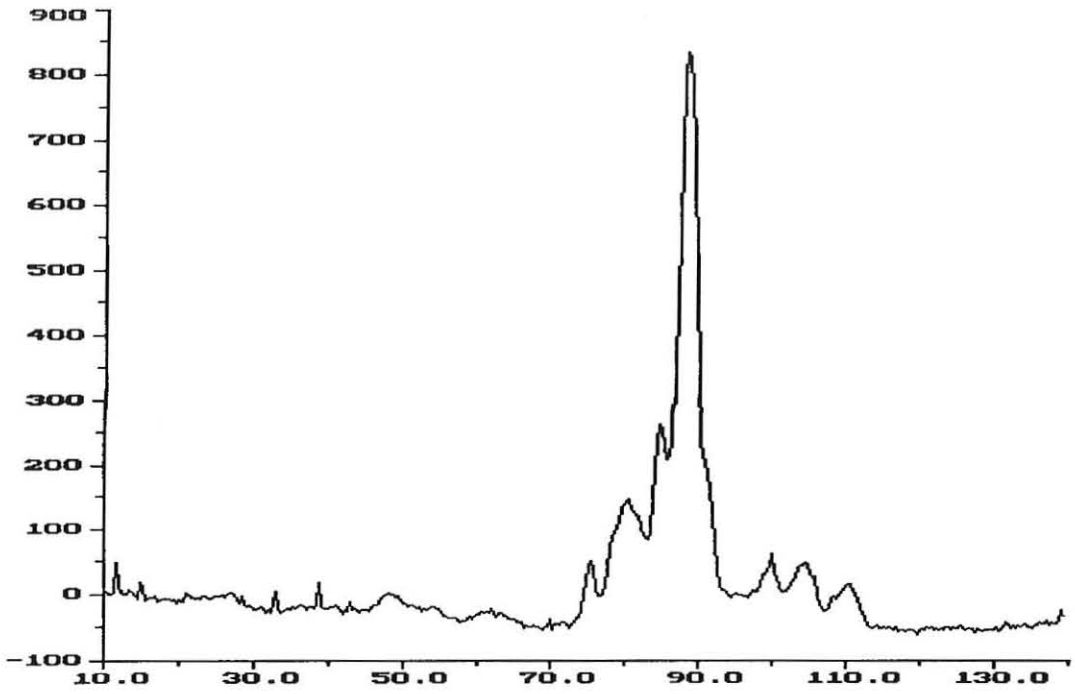


Figure 3.19: TLC-densitometry chromatogram of β-escin (mobile phase: lower phase of chloroform/methanol/acetic acid/water (63:32:12:18 v/v)).

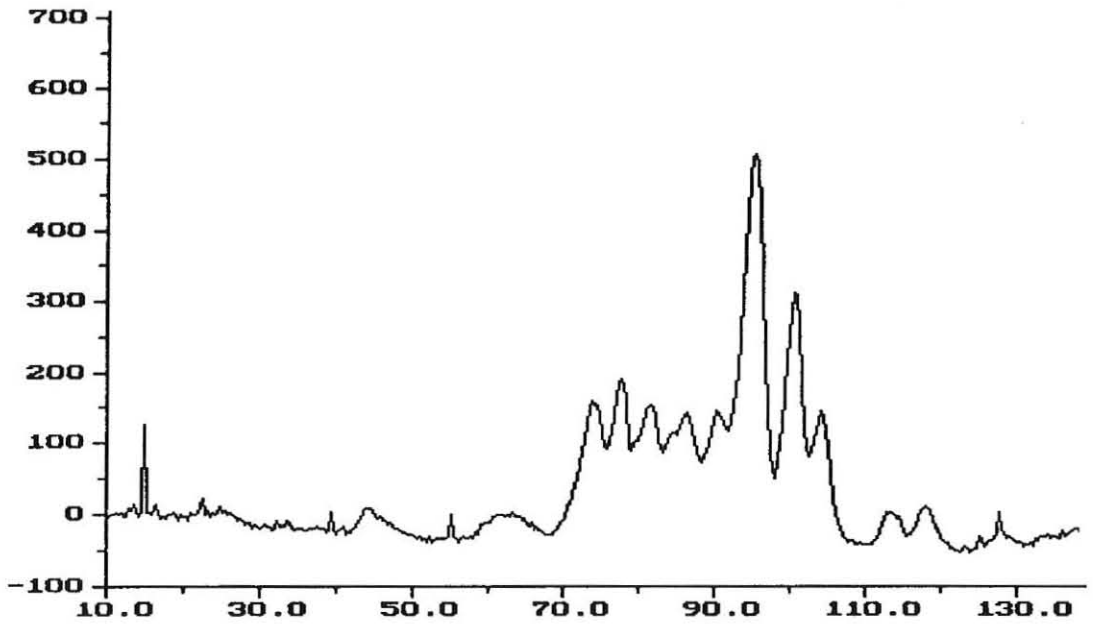


Figure 3.20: TLC-densitometry chromatogram of saponins of *G. lotoides* (mobile phase: lower phase of chloroform/methanol/acetic acid/water (63:32:12:18 v/v)).

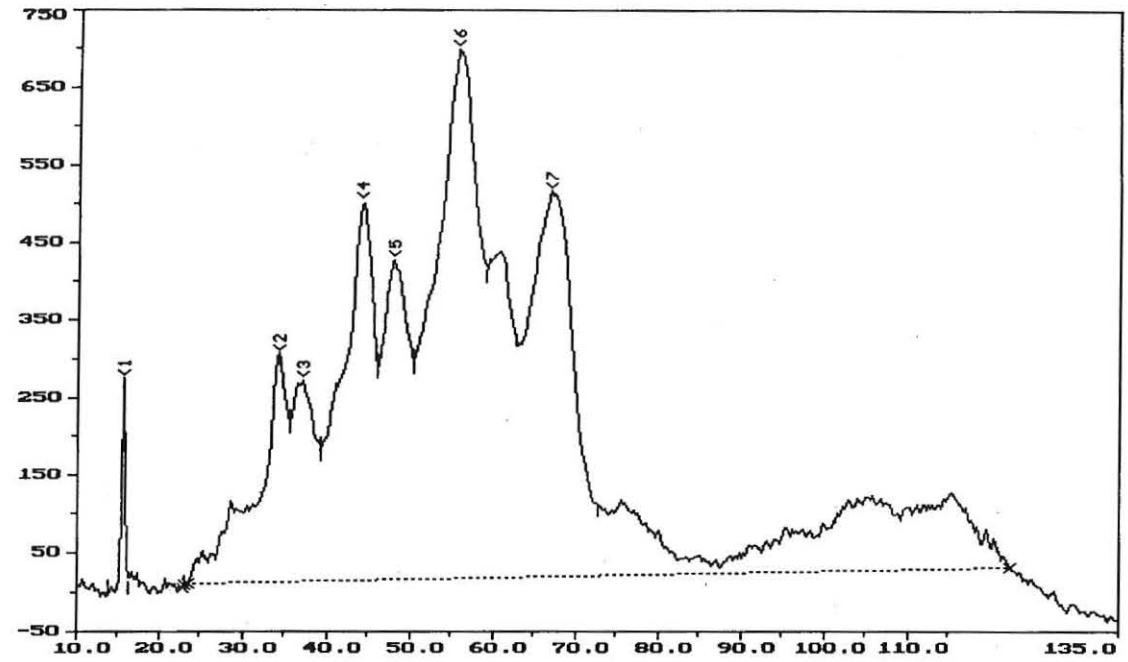


Figure 3.21: TLC-densitometry chromatogram of saponins of *G. lotoides* (mobile phase: 1,2 dichloroethane/ethanol/methanol/water (50:20:20:6 v/v)).

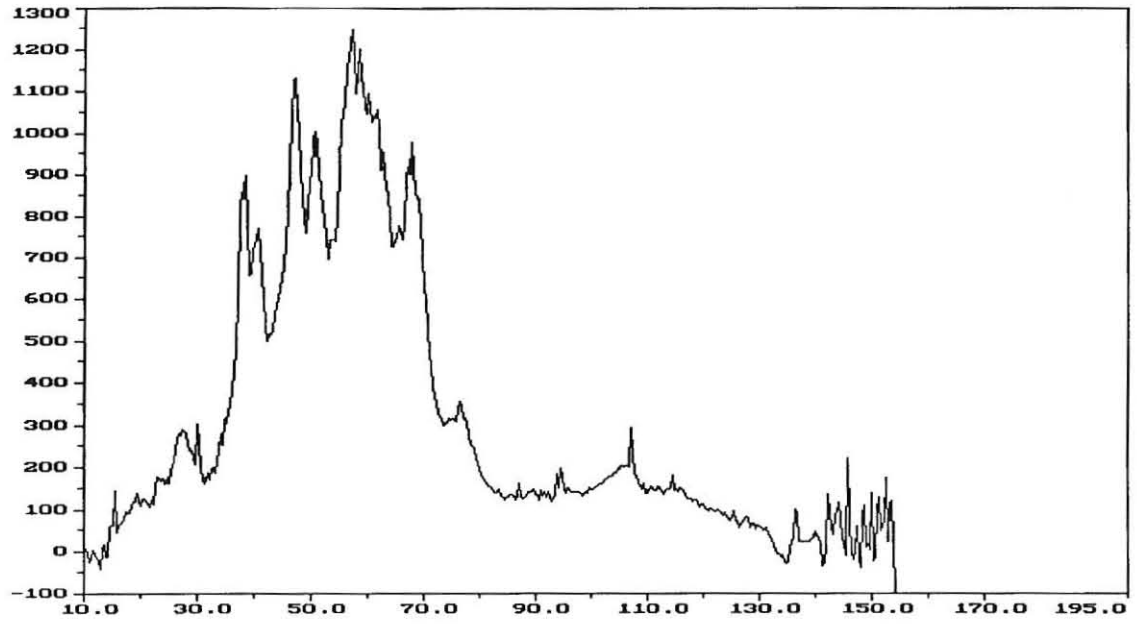


Figure 3.22: TLC-densitometry chromatogram of saponins of *G. lotoides* (mobile phase: Chloroform/methanol/water/Formic acid (50:40:8:1 v/v)).

3.3 Characterisation of the dry extract

Assessment of the physico-chemical properties of drug powder whether it is a pure chemical or an extract is a primary task during preformulation studies. Formulation of plant extract is a challenge in pharmaceutical technology due to the complex physico-chemical properties of the extract (27). Physcio-chemical properties such as, size, surface area and surface volume ratio of a particle, moisture content and densities can be related to the physcio-chemical and pharmacological properties of a drug. Moisture modifies the flow and mechanical properties of many powders particularly plant dry extracts (81). Hence, investigation of some physicochemical properties of the extract is imperative before commencing the formulation. In this respect, physical properties of the extracts of *G. lotoides*, such as particle size and size distribution, morphology, water sorption pattern, bulk, tapped and true densities, flow properties (angle of repose and flow rate) and compaction profiles were investigated. Based on the density results, physical properties like porosity, consolidation index and Hausner ratio were calculated.

3.3.1 Particle size analysis

In the manufacture of solid dosage forms, the particle size and size distribution of the drug and excipients can play a major role. This applies particularly to formulation of tablets using direct compression. Particle size may influence number of important factors that can relate to the manufacturing of pharmaceuticals. These include: a) high proportion of fines spoils the flow properties, b) fines produce dust, c) high proportion of coarse particles leads to demixing d) coarse fraction result in unevenly distributed tablets, e) with high molecular polymers, a large coarse fraction seriously delays dissolution, and f) in direct compression, the coarse particles of a binder demonstrate a

weaker binding effect (82). For these reasons, particle size and size distribution of a material have to be determined and the fine as well as the coarse fractions have to be kept as small as possible.

The particle size and size distributions of the different extracts of the seeds of *G. lotoides* were studied using Sympateic laser diffraction spectrometer. All the extracts were powdered in mortar and pestle and sieved until all particles passed though a sieve size of 355 μm. Figure 3.23 shows the particle size and size distribution of the different extracts. As shown in the figure the average particle sizes (X_{50}) of the 60%, 70% and 80% methanol crude extracts were found to be 68.39, 92.06 and 68.52 μm respectively. 60% and 80% methanol crude extracts showed similar particle size, (in fact the curves are superimposed), whereas 70% methanol showed relatively bigger particles.

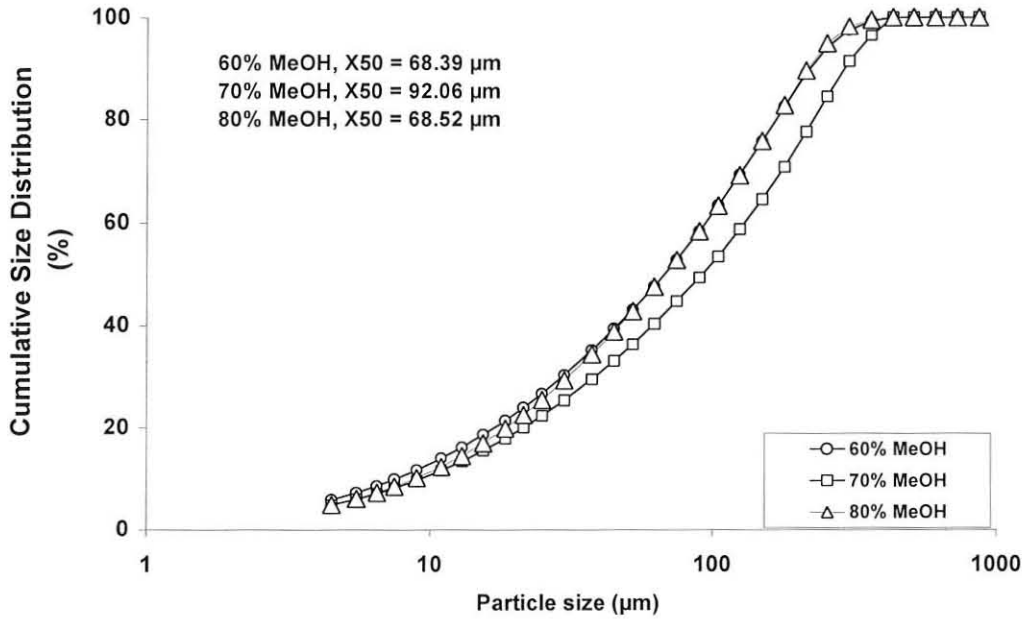


Figure 3.23: Cumulative particle size and size distribution of the powdered crude extracts of *G. lotoides*.

3.3.2 Morphology

Surface structures of the powdered extracts obtained by vacuum oven and freeze drying, the n-butanol fraction and the purified powders were determined in a scanning electron microscope (Fig. 3.24–3.29). The accelerating voltage and the magnification are shown on the micrographs.

Figure 3.24 and 3.25 show the scanning electron micrographs of vacuum oven dried crude extracts of the seeds of *G. lo*toides at magnifications of 500 and 2000 times respectively. As shown in the micrographs, the particles of the vacuum oven dried crude extracts are irregular in shape and relatively compact masses with sharp edges. On the other hand, the saponins of *G. lotoides* appear spherical, relatively regular and composed of very small particles (Fig. 3.26).

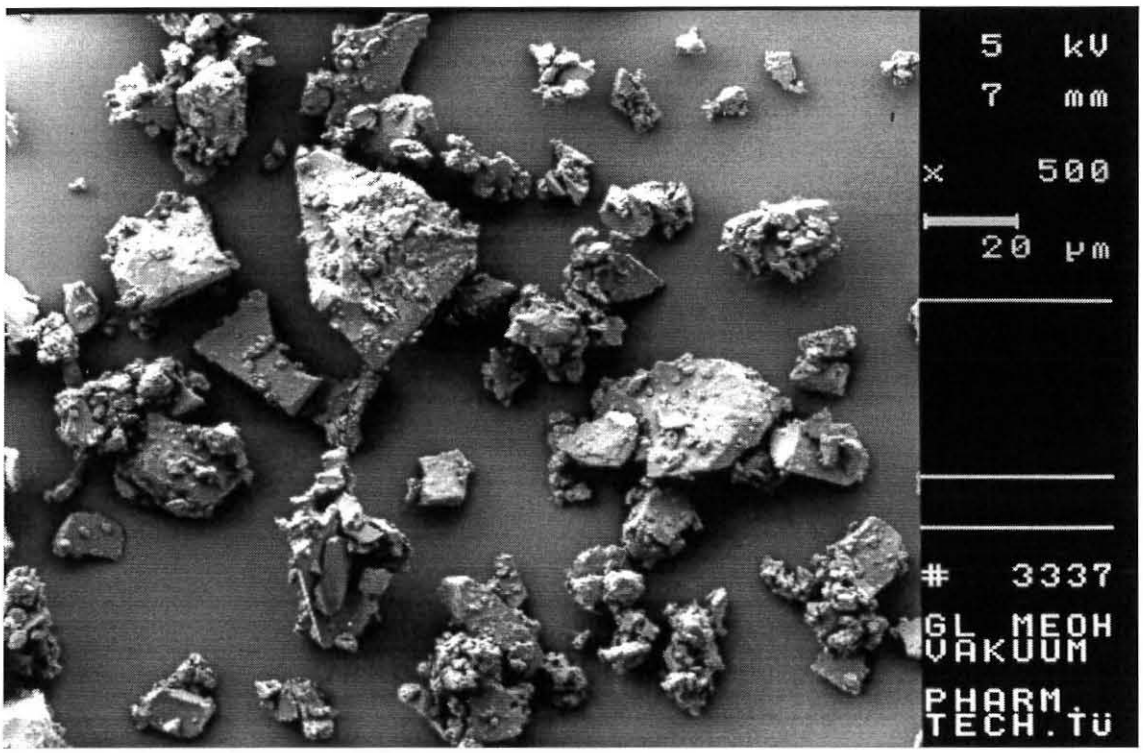


Figure 3.24: Scanning electron micrographs of crude extracts of *G. lotoides* (Vacuum oven dried, 500X).

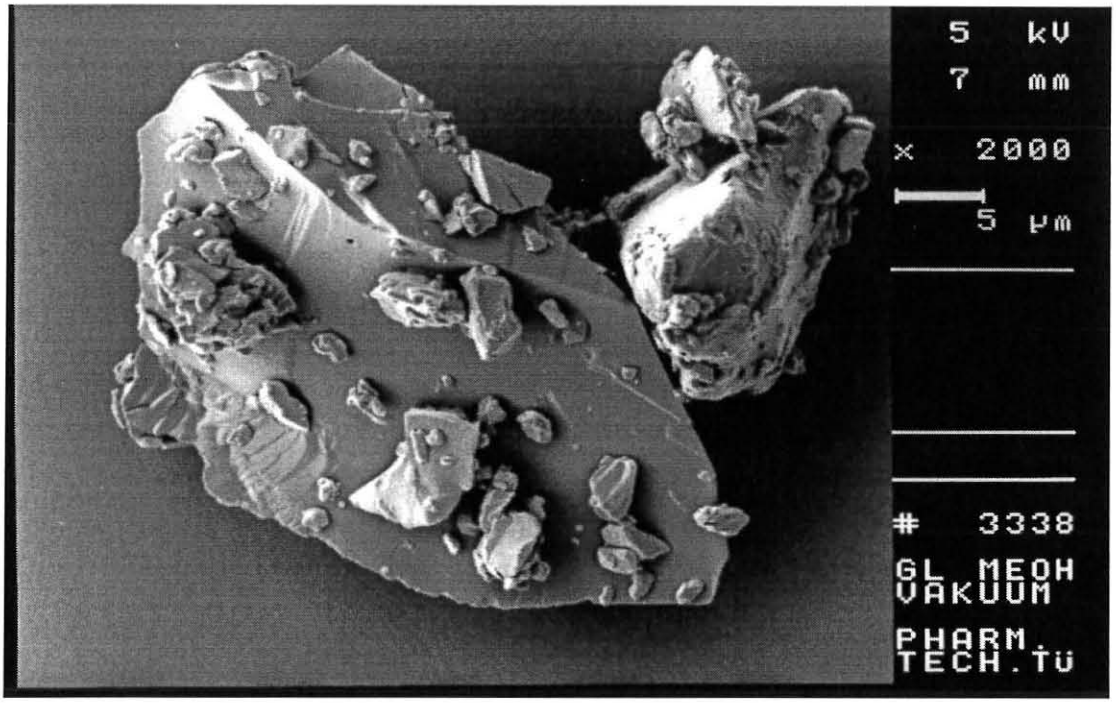


Figure 3.25: Scanning electron micrographs of crude extracts of *G. lotoides* (Vacuum oven dried, 2000X)

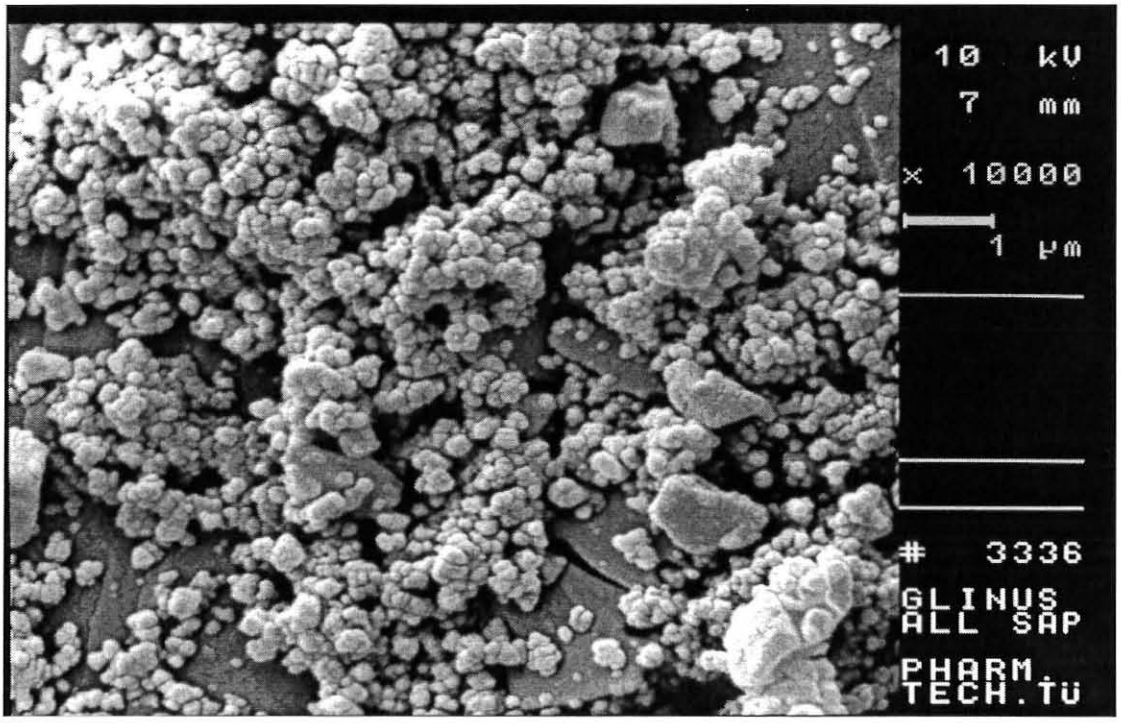


Figure 3.26: Scanning electron micrographs of saponins of *G. lotoides* (10,000X)

The micrographs of the freeze dried at various magnifications are depicted in Figure 3.27 and 3.28 and the n-butanol fraction is depicted in Figure 3.29. As shown in the micrographs, the freeze dried crude extracts are composed of irregular shape particles, which are aggregated together.

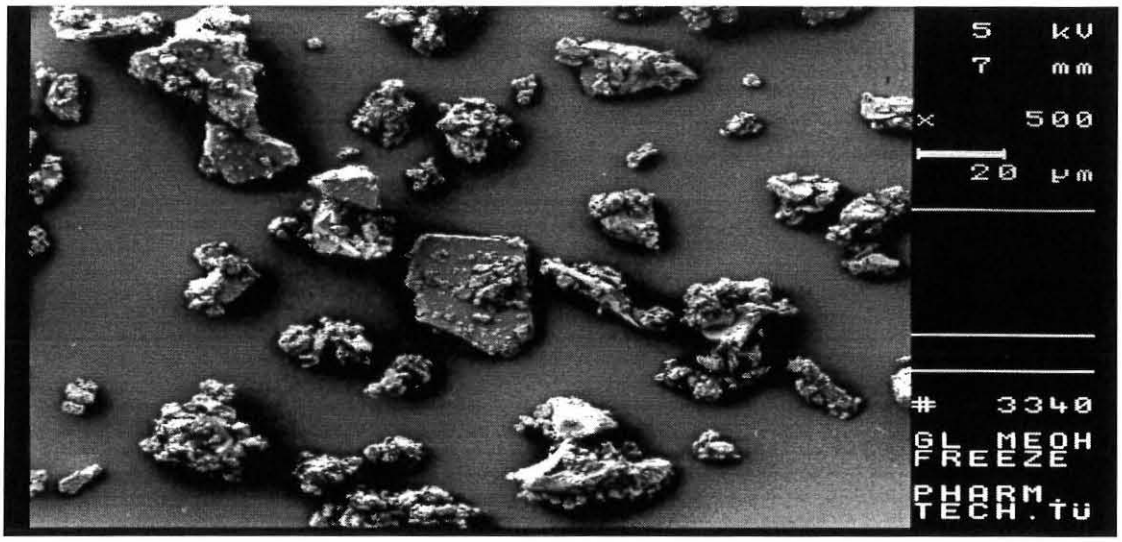


Figure 3.27: Scanning electron micrographs of crude extracts of *G. lotoides* (freeze dried, 500X).

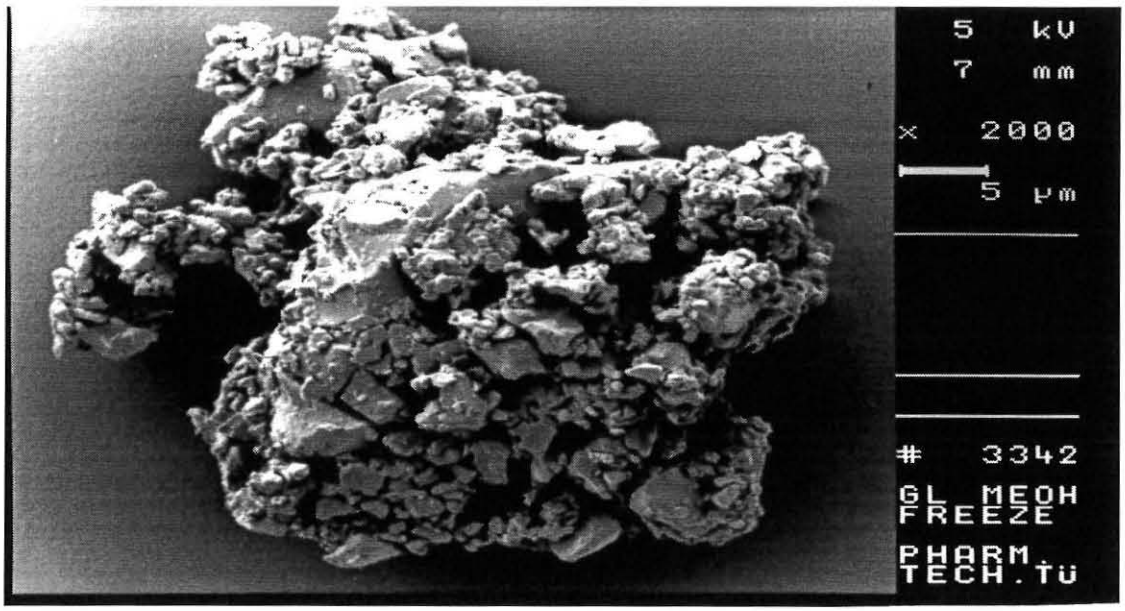


Figure 3.28: Scanning electron micrographs of crude extracts of *G. lotoides* (freeze dried, 2000X).

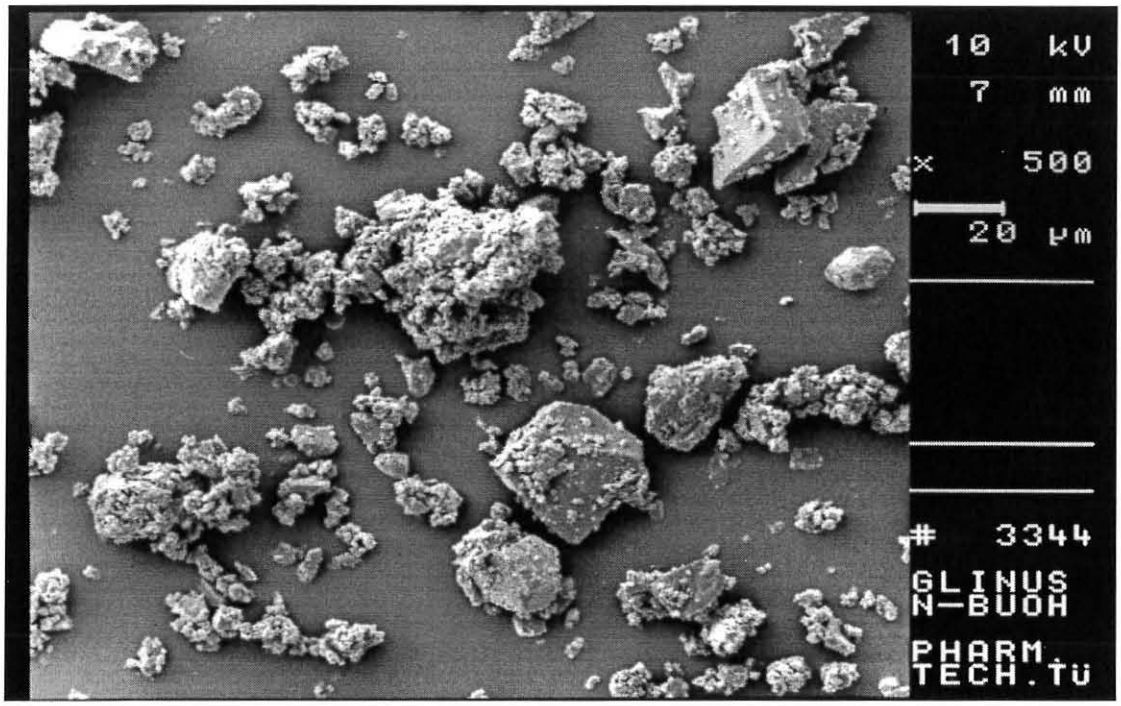


Figure 3.29: Scanning electron micrographs of n-butanol fraction of *G. lotoides* (500X).

3.3.3 Water sorption

Many drugs substances, particularly plant extracts, have a tendency to adsorb moisture. Adsorption and equilibrium moisture content can depend upon different factors such as atmospheric humidity, temperature, surface area, exposure time and the mechanism for moisture uptake. With most hygroscopic materials, changes in moisture level can greatly influence many important parameters, such as chemical stability, flow properties and compatibility (83).

Physical properties such as angle of repose, bulk and true densities and porosity of an extract were found to vary with moisture levels. The physical properties such as angle of repose, bulk densities and porosity of Kabuli chana, black pepper and pulse seeds extracts were found to be affected by various moisture levels (84–86).

Figure 3.30 shows the water uptake profiles of the freeze dried crude extracts (80% methanol) of the seeds of *G. lotoides* at different relative humidities at 20° C. As shown in the figure, the percent water uptake by the crude extract increases with increase in relative humidity. Moreover, the powdered extract requires longer time to reach maximum and equilibrium water uptake. On the other hand, purification of the crude extract has significantly reduced the hygroscopicity of the extract due to the removal of extraneous materials. As shown in Figure 3.31, the n-butanol fraction of the seeds of *G. lotoides* was found to be hygroscopic only at higher relative humidity, i. e., 85 %.

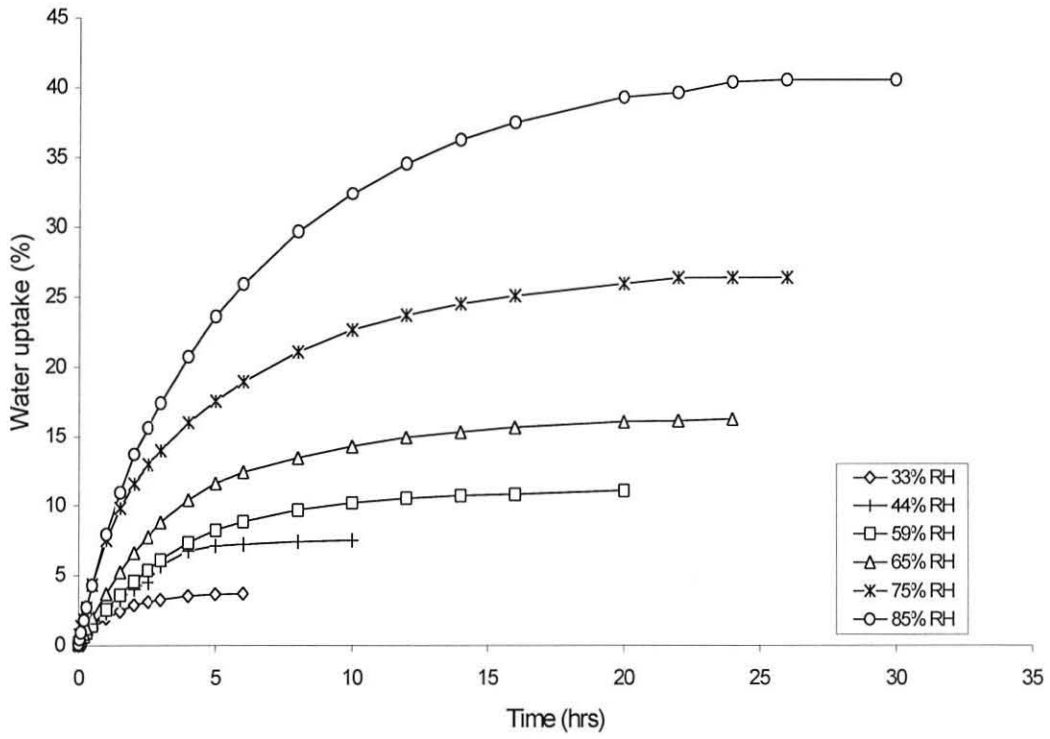


Figure 3.30: Water uptake profiles of the freeze-dried crude extracts (80% methanol) of the seeds of *G. lotoides* at different relative humidities at 20° C.

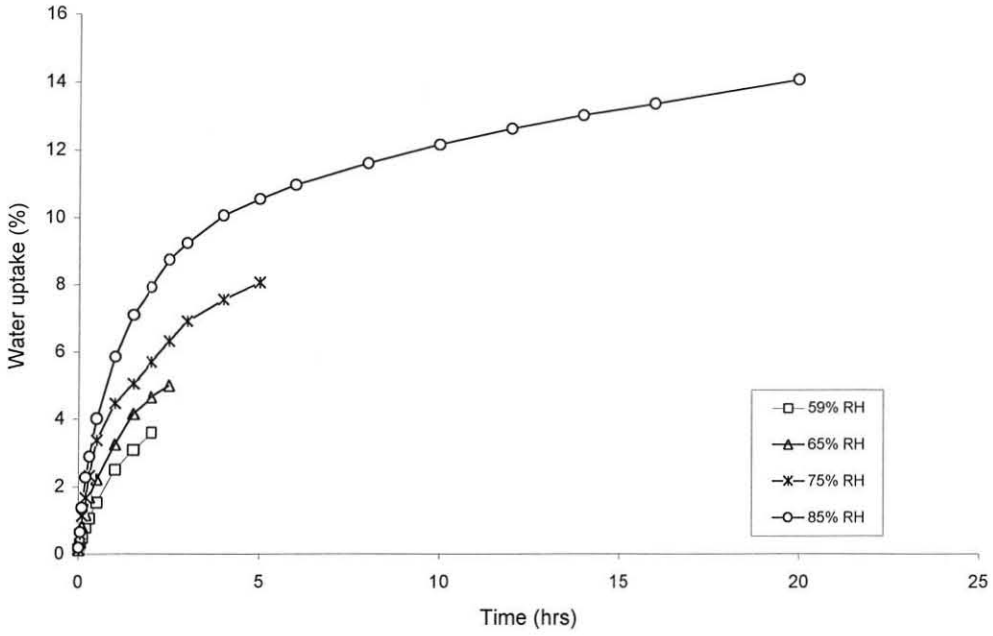


Figure 3.31: Water uptake profiles of the n-butanol extract of the seeds of *G. lotoides* at different relative humidities, at 20° C.

Water sorption isotherms of the extracts were determined by taking the equilibrium water uptake of the extracts at different relative humidities. Such representations have the advantages of indicating water uptake profile of the various extracts at different relative humidities. Based on sorption isotherm results, the necessary precautions could be taken during manufacturing and storage.

Figure 3.32 shows the water sorption isotherms of the freeze and vacuum oven dried crude extracts, n-butanol fraction and the purified extract of the seeds of *G. lotoides* at 20° C. As shown in the figure 3.32, purification of the extract to n-butanol fraction or saponins of *G. lotoides* decreases the hygroscopicity. Moreover, the sorption isotherms

of the crude extract show a marked water uptake increase after 60% relative humidity, indicating that, the hygroscopicity of the crude extracts are manageable at lower relative humidities.

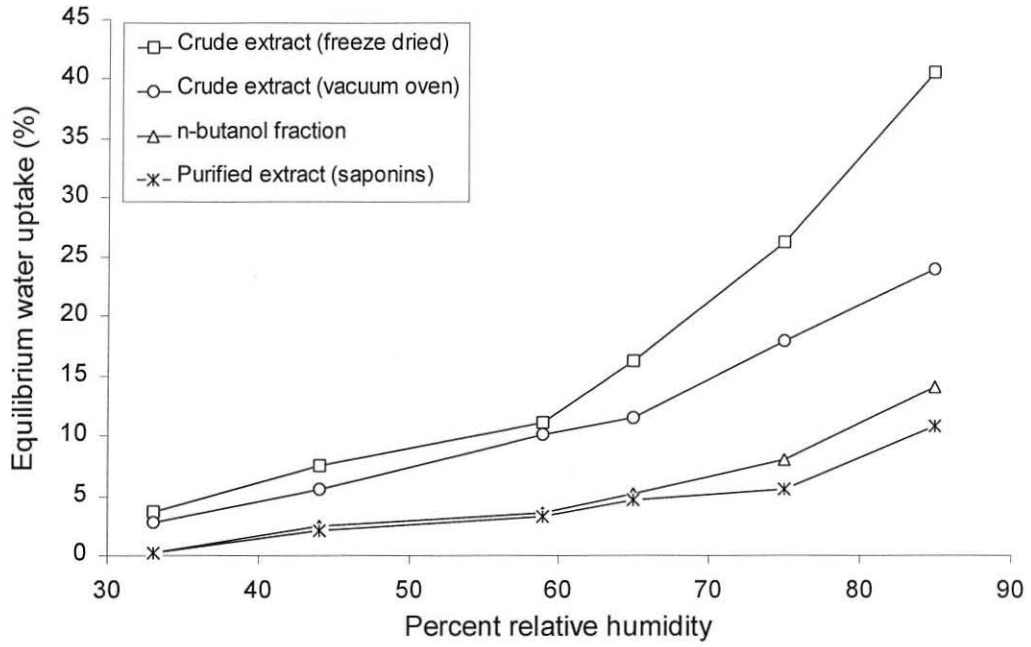


Figure 3.32: Water sorption isotherms of the freeze dried and vacuum oven dried crude extracts, n-butanol fraction and the purified extract of *G. lotoides* at 20° C.

The physical properties of dry extracts, in addition to other factors, depend on the drying process. Based on the method of treatment, the morphology and the surface area of the extract could vary. The effect of drying process on the water sorption profiles of the crude extracts as depicted in Figure 3.32 and 3.33. As shown in the figures, the freeze-dried crude extracts are more hygroscopic than the vacuum oven dried, particularly at higher relative humidity. The higher water sorption properties of freeze-dried extracts may be attributed to the higher surface area of these products and this have been also mentioned elsewhere (87,88).

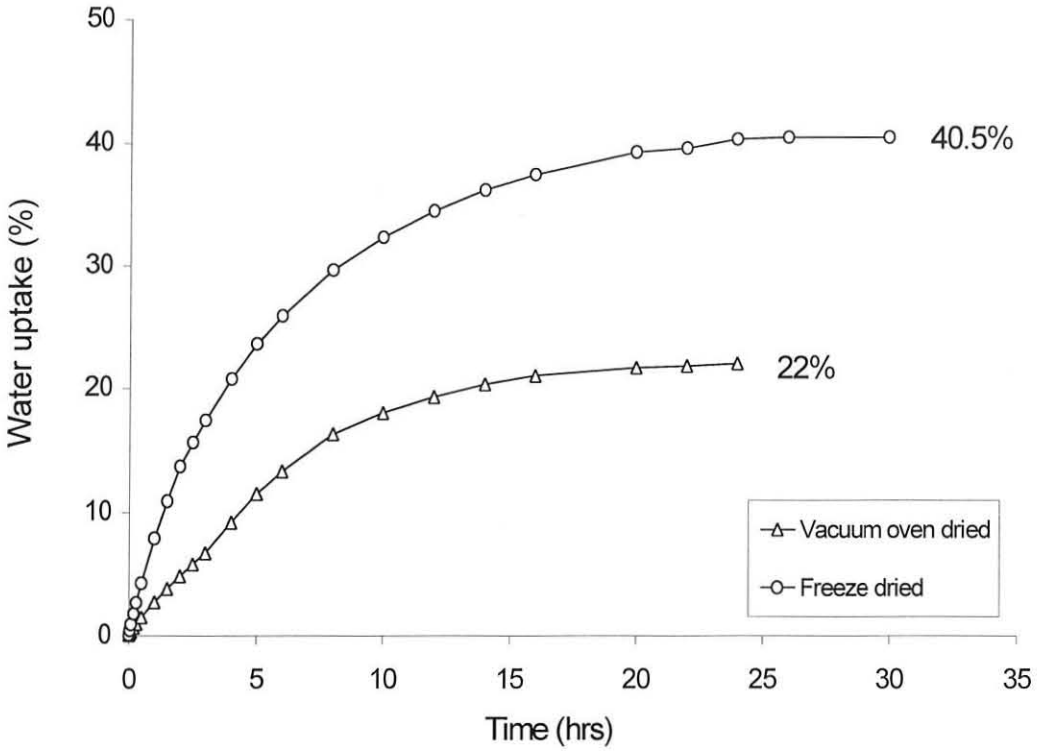


Figure 3.33: Water uptake profiles of the freeze dried and vacuum oven dried crude extract of the seeds of *G. lotoides* at RH 85% and 20° C.

Water sorption profiles of the various crude extracts of the seeds of *G. lotoides* obtained using 60%, 70% and 80 % methanol were determined (Fig. 3.34). As shown in Figure 3.34, the hygroscopicity of the different crude extracts differs mainly at higher relative humidities, namely at 75% and 85%. Moreover, the hygroscopicity of the crude extracts increases with decrease in methanol ratio of the extracting solvent. Hence, 60% methanol crude extract is more hygroscopic than 70% and 80%. These could be attributed to the presence of more water-soluble compounds in crude extracts of 60% than those containing lower percentage of water (70% and 80% methanol).

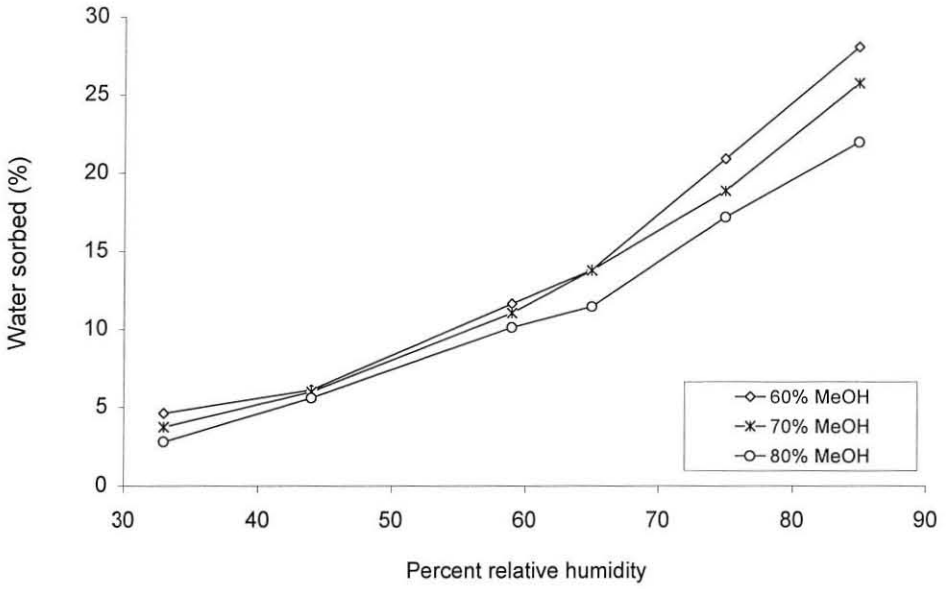


Figure 3.34: Water sorption isotherms of the various crude extracts of the seeds of *G. lotoides* (vacuum oven dried) at 20°C.

Analytical methods for monitoring the moisture level of extracts include: gravimetry (an adsorption isotherm measurement with a gravimetric sorption analyser (GSA)), Thermal Gravimetric Analysis (TGA), Karl Fischer Titration or Gas Chromatography. Each method has it own advantage depending on the desired precision or the amount of moisture adsorbed onto the drug sample (89,90).

In this study, the water content of the various crude extracts of the seeds of *G. lotoides* was determined using Karl Fischer titration. The results indicate that, the crude extracts contain 3.63% (S.D. 0.11), 3.5% (S.D. 0.13) and 3.36% (S.D. 0.14) water for 60%, 70% and 80% methanol crude extracts respectively.

3.3.4 Densities

The density of solids corresponds to their average mass per unit volume and typically is expressed in grams per cubic centimetre (g/cm^3). Unlike gases and liquids whose density depends only on temperature and pressure, the density of a solid particle depends on its molecular assembly and therefore varies with the crystal structure and degree of crystallinity. When a solid particle is amorphous or partially amorphous, its density may further depend upon the history of preparation and treatment. Therefore, unlike fluids, the densities of two chemically equivalent solids may be different, and this difference reflects a difference in solid-state structure (91).

Furthermore, the density of particles is an important physical characteristic of pharmaceutical powders. Uniformity of dosage, powder flow property and compressibility can be affected by variation in density. The density of a solid particle can assume different values depending on the method used to measure the volume of the particle. It is useful to distinguish three levels of expression of density. These are crystal density (true density), particle density (granule density) and bulk density (91).

Bulk density of a powder bed is not uniform and hence the physical property of the bed is not uniform either. The bulk density of a powder includes the contribution of interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the space arrangement of particles in the powder bed. The bulk density of a powder is often very difficult to measure since the slightest disturbance of the bed may result in a new density value. Thus, it is essential in reporting bulk density to specify how the determination was made (91).

Table 3.3 shows the densities of the different crude extracts of the seeds of *G. lotoides*. The various crude extracts have similar bulk and true densities. However, the 60% MeOH crude extract show a higher tapped density indicating that it may show a better compressibility than 70% and 80 % MeOH crude extracts.

Table 3.3: Densities of the crude extracts of the seeds of *G. lotoides.*

Crude extract of *G. lotoides*	Densities (g/ml)		
	Bulk density	Tapped density	True density
60 % MeOH	0.66	0.94	1.51
70 % MeOH	0.67	0.85	1.49
80 % MeOH	0.67	0.84	1.50

Density is directly proportional to porosity. Porosity and consolidation index, which are measures of compressibility of powders were calculated from the density results. Thus, porosity, consolidation index (Carr index) and the Hausner ratio were calculated using the following equations (81,92,93).

$$\text{Porosity (e)} = \frac{\text{Bulk volume} - \text{True volume}}{\text{Bulk volume}} \quad (3.1)$$

$$\text{Consolidation index \% (Carr index)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \quad (3.2)$$

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad (3.3)$$

Table 3.4 shows the results of porosity, consolidation index and Hausner ratio. As shown in the table, 60% MeOH crude extract exhibited a better compression property and higher porosity than 70 % and 80 % MeOH crude extracts.

Table 3.4: Porosity, consolidation index and Hausner ratio of the crude extracts of the seeds of *G. lotoides*

Crude extract of *G. lotoides*	Porosity (%)	Consolidation (Carr's) index (%)	Hausner ratio
60 % MeOH	56.0	29.8	1.42
70 % MeOH	55.4	21.2	1.27
80 % MeOH	55.5	20.2	1.25

Carr's index is a simple index that can be determined on small quantities of powder. It may be provide information about the powder flow properties. Hence, Carr's index (%) 5-15 is considered as excellent, 12-16 good, 18-21 fair (passable), 23-35 poor, 33-38 very poor and greater than 40 extremely poor powder flow properties (92). Accordingly, as shown in Table 3.4, the crude extracts of the plant showed Carr's index in the range of poor flow properties.

Another similar index that indicates powder flow property is Hausner ratio. Values less than 1.25 indicate good flow, while greater than 1.25 indicates poor powder flow properties (93). The Hausner ratio of the crude extracts (Table 3.4) also showed that the extracts have a poor flow property.

Compressibility is the ability of a powder bed to be compressed, i. e, reduced in volume due to the application of a given stress. Consolidation is a term mostly used as a

synonym with compaction and used to describe compression properties of powders. Carr's index is a one-point determination and does not always reflect the case or speed with which consolidation of powder occurs. In deed some materials have a high index (suggesting poor flow) but they may consolidate rapidly. Rapid consolidation is essential for uniform filling on tablet machines (93,94).

3.3.5 Flow properties

One of the properties of powders which is of great concern to pharmacists is the extent to which free flow will occur. The control of weight variation of tablets is dependent upon the reproducibility of powder flow into the dies. The flow properties of powders vary considerably, ranging from those that flow readily and uniformly to those that resist free flow. Particulate solids resist flow because of the frictional and cohesive forces (95).

Angle of repose and flow rate (timed delivery from an orifice) are methods that are commonly used to evaluate powder flow properties. As correlation between these two methods does not always exist, (96-98) both properties were determined. The frictional and cohesive forces in powder are reflected in the angle of repose. Angle of repose is defined as the maximum angle "θ" between the surface of the powder pill and the horizontal plane, and calculated using the equation:

$$\tan \theta = h/r \qquad (3.4)$$

where 'h' is height and "r" is radius of the powder pill. The values of angle of repose obtained may be used as a guide to the type of flow expected. Angle of repose less than 25° indicates the powder has excellent flow properties, whereas 25°-30° good, 30°-40° passable (fair) and greater than 40° very poor (93).

Table 3.5 shows the flow properties of the crude extracts of the seeds of *G. lotoides*. As shown on the table, all the crude extracts show poor flow properties. A number of reasons could be cited for the poor flow properties exhibited by the crude extracts. Particle shape and surface texture are known to have an important influence. As shown in Figure 3.24 and Figure 3.25, the crude extracts are composed of rough irregular particles, which could present more points of contact and hence higher friction and resulting in less free flowing property. Another factor is the particle size and size distribution. As shown in Figure 3.23, the crude extracts contain very fine particles, which prevent or hinder flow because fine particles lead to packing and powder densification.

Table 3.5: Flow properties of the crude extracts of the seeds of *G. lotoides.*

Crude extract of *Glinus lotoides*	Angle of repose	Flow rate (g/sec)
60 % MeOH	43.7°	Doesn't flow
70 % MeOH	46.0°	Doesn't flow
80 % MeOH	44.6°	Doesn't flow

3.3.6 Compactibility

Compactibility is defined as the ability of a powder bed to cohere into or to form a compact mass. Compactibility is usually described in terms of tablet strength as a function of applied compaction stress (94).

Figure 3.35 shows the compaction profiles of the different crude extracts of the seeds of *G. lotoides*. The extracts were compressed at different compression forces after mixing with Lubricant A. As shown in the figure, 80% MeOH crude extract show higher compactibility. The profile for 80% MeOH crude extract exhibited an increase in hardness of the tablet with increase in compression force. This high compression property of the 80% MeOH crude extract may be attributed to the presence of some oils and fats, which may contribute to the binding properties of this material. On the other hand, 60% and 70% MeOH crude extracts showed comparable compactibility, which is still hard enough to form compact masses. The difference in compaction profiles among the crude extracts may be due to the variation of their composition, for instance the amount of extraneous material, which probably resulted in different compaction mechanism. However, further studies have to be conducted to substantiate this assumption.

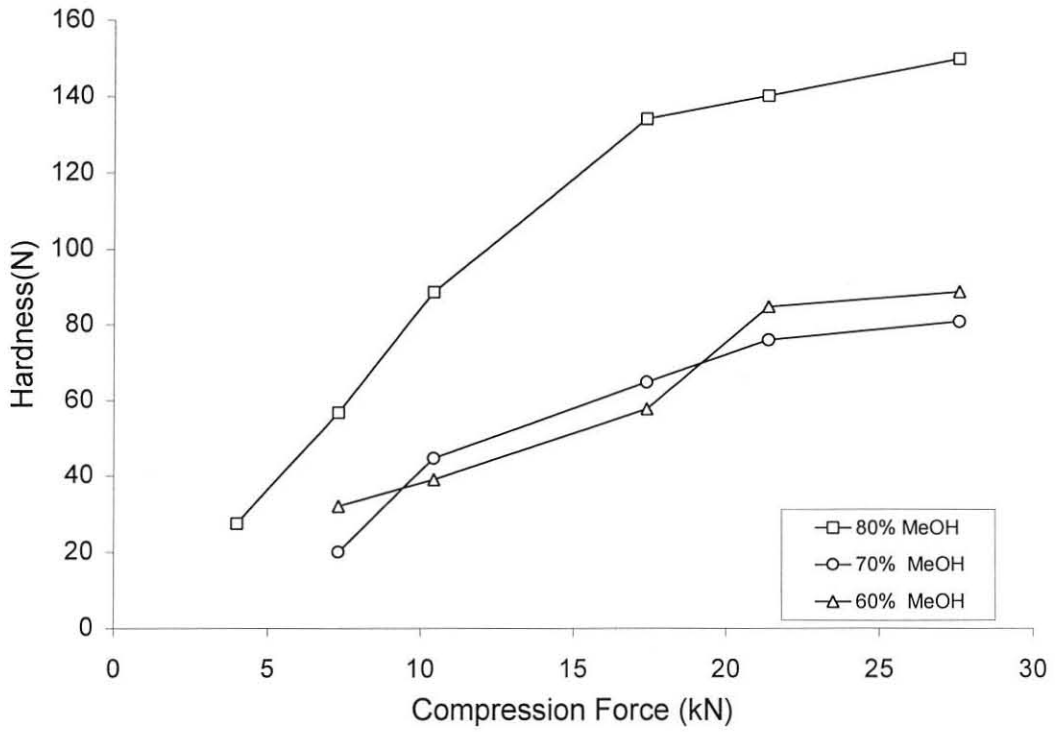


Figure 3.35: Compaction profiles of the crude extracts of *G. lotoides*

Angle of repose and Hausner ratio measurements indicate a correlation between the internal forces of friction and cohesion of the sized powders and the tensile strength of compacts formed from them (99). Hausner ratio and compressibility results (Table 3.4 and Fig. 3.35) of the crude extracts were indicate that 60% MeOH crude extract have a better compressibility. However, the compaction study favours 80% MeOH crude extracts. On the other hand, the compression profiles of 60% and 70% MeOH crude extracts showed a different compaction profiles that cross each other as the compression force increases. This indicates that various processes such as elastic deformation, plastic and fragmentations, take place during compaction and sometimes it is difficult to conclude based on a specific property.

3.4 Preparation and evaluation of tablets

Compacted tablets are produced from granulations or powder mixtures made by the following general techniques: wet granulation or wet massing combined with tray drying or fluid-bed drying, wet granulation and drying all in one step, dry granulation by roller compaction or slugging, and direct compression (dry blending). Of these, the oldest is the classical wet granulation and drying technique in two steps using tray drying. In spite of enormous improvements in wet granulation techniques (high shear granulation, fluid-bed granulation, extrusion granulation, continuous granulation, and all-in-one granulation), tablet production by direct compression has increased steadily over the years because, it offers economic advantages through its elimination of the wet granulation and drying steps. The granulation technique that uses slugging or roller compaction is no longer a method of choice to produce compressed tablets (94).

Based up on the results of the preformulation studies, the most suitable extract of the seeds of *G. lotoides* (Extract A) was formulated and compressed into 1.15g oblong tablets (having long and short diameters of 20.1 and 8.2 mm respectively). 570 mg of Extract A was found to be equivalent to the traditional dose and this amount was formulated into tablets. The formulation containing the most suitable extract and excipients is shown in Table 3.6.

Effects of four different types of direct compression filler/binders (coded as Filler/Binder A, B, C, and D) and two disintegrants (coded as Disintegrant A and B) on hardness and disintegration times of *G. lotoides* tablets were evaluated. Glidant A and Lubricant A were also included in the formulations.

Table 3.6: Formulations of *G. lotoides* extract containing various types of filler/binder and disintegrants.

Ingredients	Amount (mg)
Extract A	570
Filler/binder (A, B, C or D)	511
Disintegrant (A or B)	57.5
Glidant A	5.75
Lubricant A	5.75

The crushing strength of the tablets was determined to compare the relative hardness of the tablets and was measured after 24 hrs to allow for completion of elastic deformation and particle rearrangement (consolidation).

Figure 3.36 shows the effect of the filler/binders and disintegrants on hardness of *G. lotoides* tablets prepared at 15 kN. As shown in the figure, Filler/Binder B and C, produced harder tablets with crushing strengths greater than 250 N at 15 kN. On the other hand, Filler/Binder A and D formed tablets with lower crushing strengths but with sufficient strength. Effect of disintegrant type on the hardness of tablets were not significant except in the formulation that contains Filler/Binder B. In this, formulation containing Disintegrant A showed higher crushing strength than Disintegrant B.

The effect of compression force on tablet properties; namely crushing strength, disintegration times and friability, was evaluated by compressing a formulation containing Filler/Binder A and Disintegrant A at approximately 6.5, 8.4, 12.1, 15.5, 18.4, and 22.8 kN.

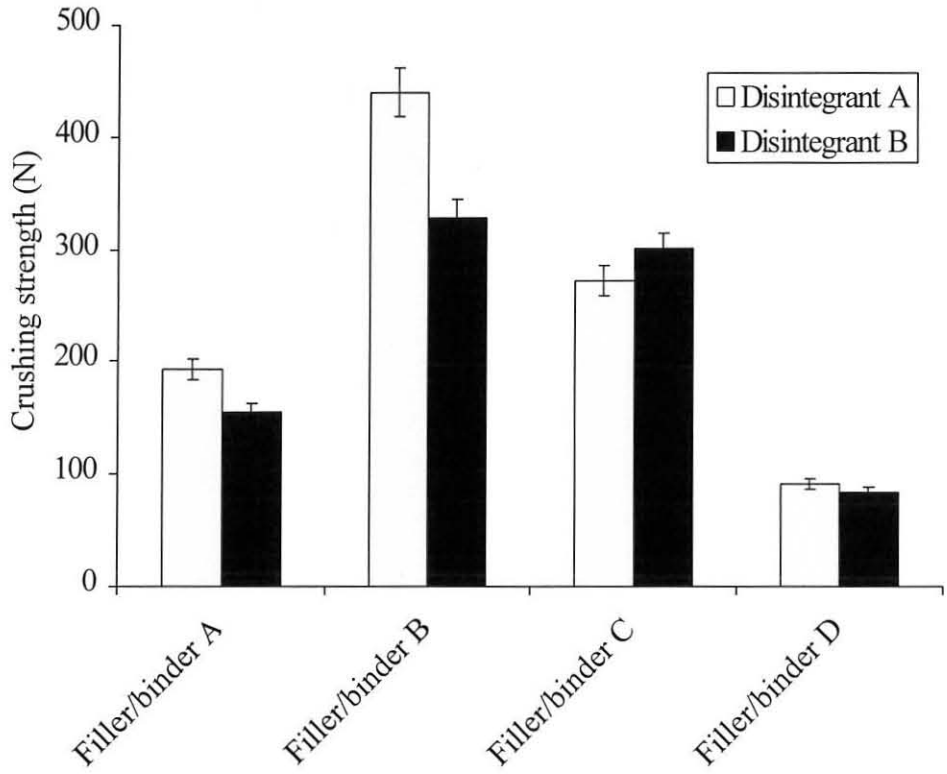


Figure 3.36: Effect of filler/binder and disintegrant on the crushing strengths of *G. lotoides* tablets.

Figure 3.37 shows the effect of filler/binder and disintegrant on disintegration time of *G. lotoides* tablets. Disintegration of a compressed tablet is the first step and it may also be the limiting step for the dissolution of active ingredients. As shown in Figure 3.37, tablets prepared from Filler/Binder A and D showed faster disintegration than those prepared form Filler/Binder B and C.

Figure 3.37 also depicts the effect of disintegrant type on the disintegration times of the tablets. It has been reported that the efficiency of disintegrant is strongly dependent on the nature of the filler/binder (100).

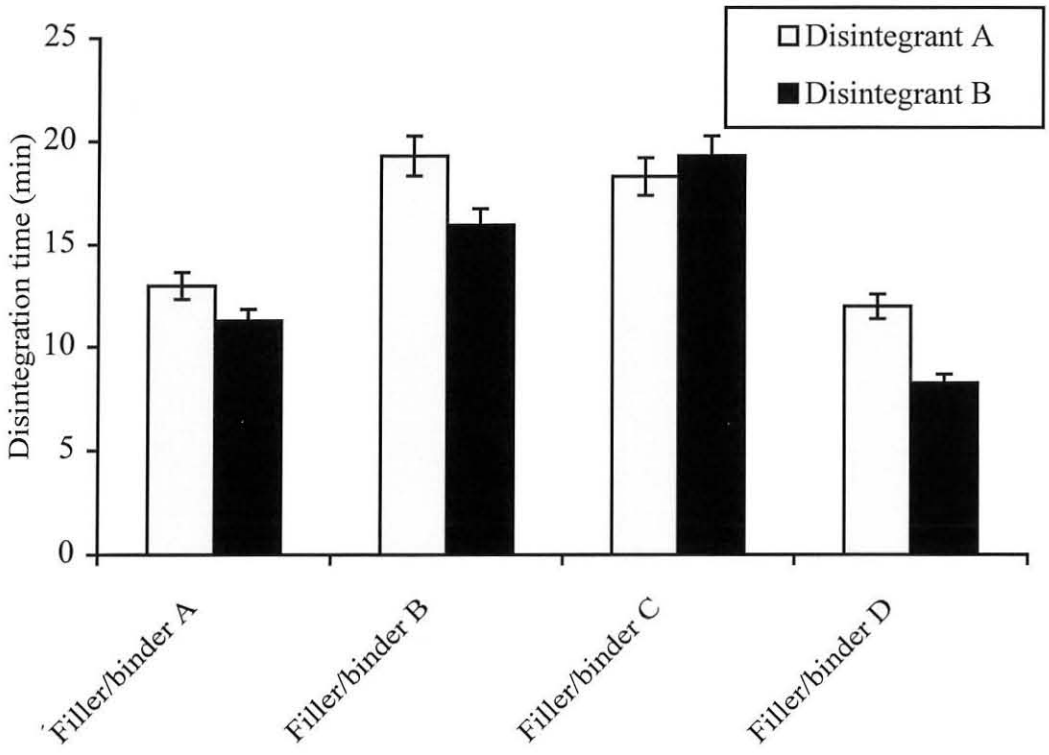


Figure 3.37: Effect of filler/binder and disintegrant on disintegration time of *G. lotoides* tablets.

As shown in the figure, tablet formulations containing Disintegrant B and Filler/Binder B and D exhibited faster disintegration times than those tablets containing Disintegrant

A and same Filler/Binders (B and D). On the other hand, both disintegrants showed similar disintegration time in formulations containing Filler/Binder A and C.

Mechanisms of tablet disintegration vary with the type of disintegrant and the formulation processes used. Different mechanisms of disintegrations, such as swelling of the disintegrant particles, capillary action of the porous network, the heat of wetting, wicking and deformation of tablets have been reported (101-103). As shown in Figure 3.37, irrespective of the type of disintegrants, the tablets showed slower disintegration times. The tablets exhibited surface erosion (i.e., dissolution) rather than spontaneous disintegration. This phenomenon could be explained or attributed to the presence of hygroscopic extract in the formulation. The delaying effect of hygroscopic ingredients on the disintegration times of tablets containing super disintegrants has been reported (104). This is so, because the hygroscopic ingredients promote dissolution rather than disintegration of the tablet. The rate of water penetration into tablets was also shown to decrease as the solubility of the drug increased due to competitive inhibition of the disintegrant for locally available water (105). The results of the present study are also in good agreement with previous works (104,105).

The effect of compression force on crushing strengths, disintegration times and friability of *G. lotoides* tablets was investigated using tablet formulation containing Filler/Binder A and Disintegrant A.

Figure 3.38 shows the effect of compression force on hardness of *G. lotoides* tablets. As shown in the figure, the crushing strength of tablets increased with increase in compression force. The profile of the curve indicates that, there is a tendency of

continuous increase in compactibility of the tablets. However, increase in compression force resulted in harder tablets that disintegrate slowly (Fig. 3.39). As shown in the figure, the disintegration times of the tablets was increased with compression forces. This could be attributed to the increased hardness and decreased of porosity of the tablets with increased compression force.

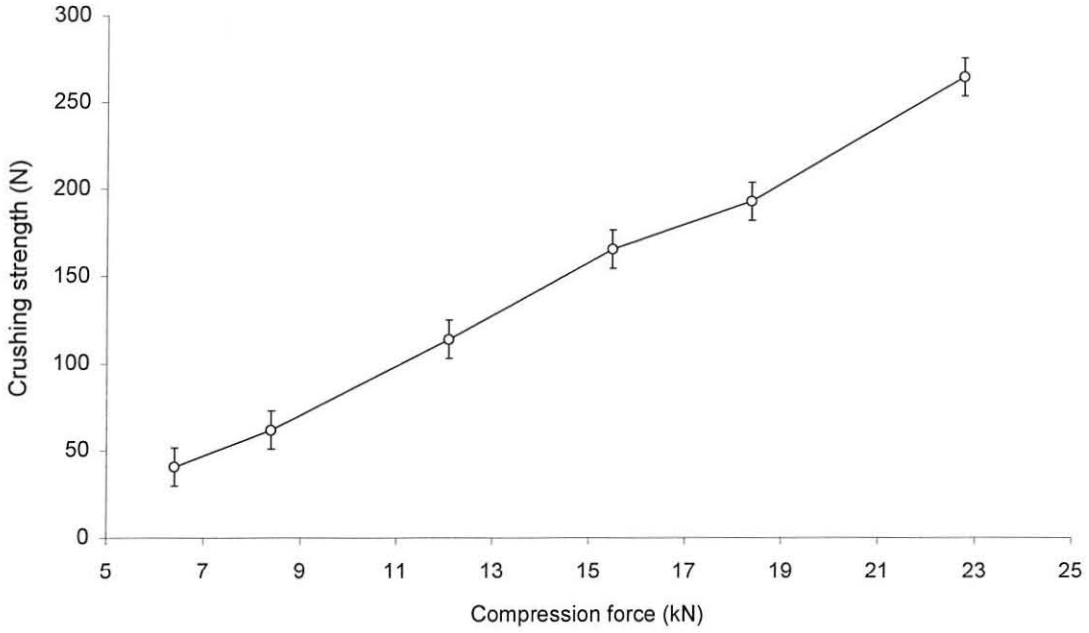


Figure 3.38: Effect of compression force on the crushing strengths of *G. lotoides* tablets.

Figure 3.40 shows the effect of compression force on the friability of *G. lotoides* tablets. As shown in the figure, the percent friability decreases with increase in compression force, obviously, due to formation of compact mass. However, even at lower compression forces, tablets of *G. lotoides* showed friability less than 1%, which is pharmacopoeia acceptable range.

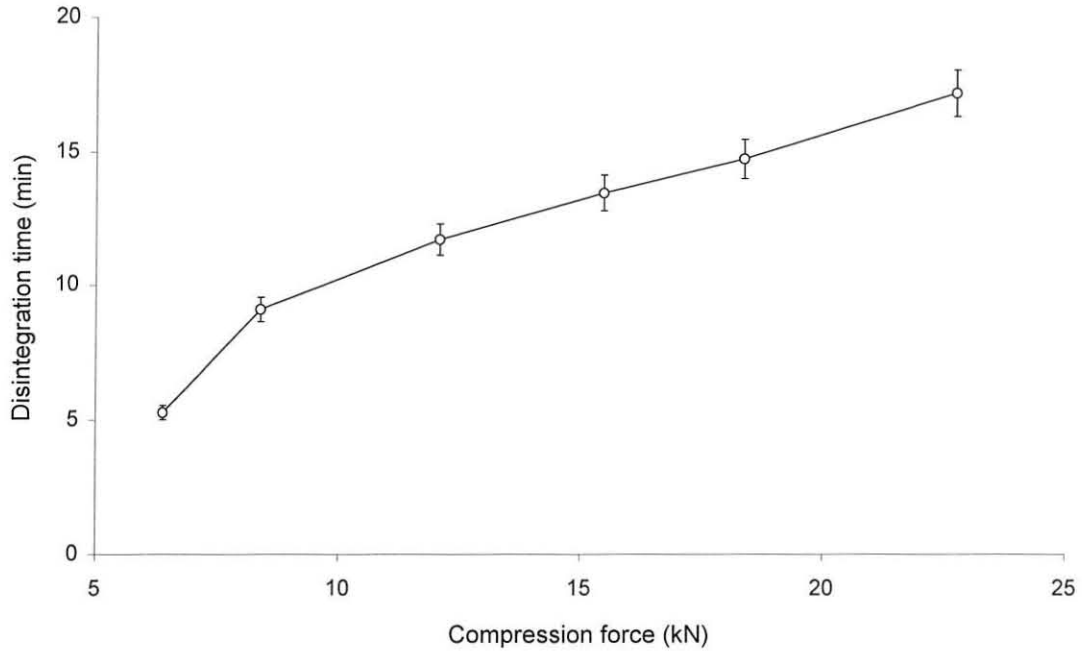


Figure 3.39: Effect of compression force on the disintegration times of *G. lotoides* tablets.

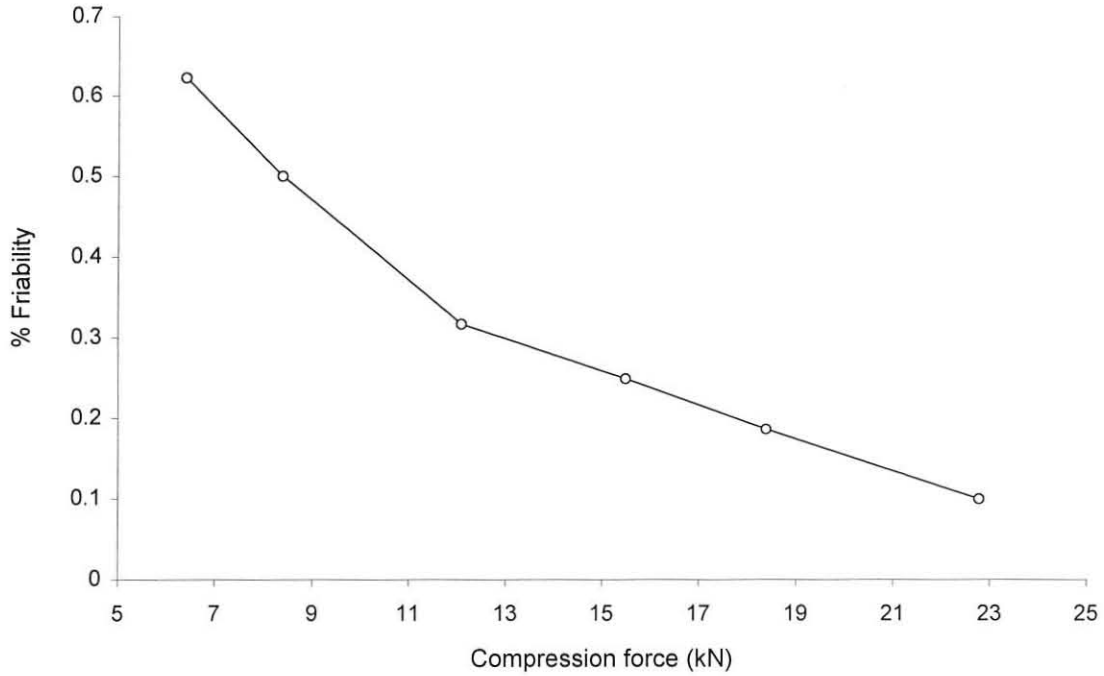


Figure 3.40: Effect of compression force on the friability of *G. lotoides* tablets.

4. CONCLUSIONS

Based on the results of the study, it can be concluded that

a) The method of extraction and purification of saponins developed are convenient, simple, efficient and reproducible.

b) The solvent system developed for extraction of saponins of *G. lotoides* provided maximum yield.

c) Colorimetry and/or UV-spectrophotometry can be utilized for quantitative measurements of saponins of *G. lotoides* in the crude extracts.

d) The physico-chemical properties of powdered extracts such as particle size and size distribution, morphology, water sorption pattern, densities, flow properties and compaction profiles depend on the nature of the extracting solvent, drying method and relative humidities.

e) When the dry crude extracts are compressed, tablet properties such as hardness, friability and disintegration times are dependent on types of filler/binder and disintegrants as well as compression force employed.

SUGGESTIONS FOR FURTHER WORK

The results of the present study lead to the following suggestions for further work.

a) Preparation and evaluation of liquid formulations.

b) Accelerated and long term stability studies and determination of shelf-life and storage conditions of dosage forms.

c) Compatibility studies of the extract with pharmaceutical excipients and containers.

d) Clinical trails of dosage forms.

e) Isolation and characterisation of the major saponin components of the seeds.

f) Total characterisation of the chemistry of the traditional dose (i.e., whole extract of the seeds of the plant).

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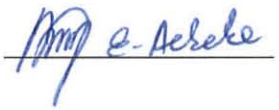
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DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university.

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