East West University

# Phytochemical and Biological Investigation of *Arachis hypogaea*

A thesis report submitted to the department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the degree of B. Pharm

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**Endorsement by the Chairperson**

This is to certify that the dissertation, entitled **"Phytochemical and Biological Investigation of *Arachis hypogaea*"** Is a thesis work done by Nazia Afrin (ID: 2012-1-70-047) in partial fulfillment of the requirements for the degree of B.Pharm. We further certify that all the sources of information and laboratory facilities availed in this connection is duly acknowledged.

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This is to certify that the dissertation, **"Phytochemical and Biological Investigation of *Arachis hpogaea*"** is a thesis work done by Nazia Afrin (ID: 2012-1-70-047), in partial fulfillment of the requirements for the degree of B.Pharm. We further certify that all the sources of information and laboratory facilities availed in this connection is duly acknowledged.

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**Declaration by the Candidate**

I, Nazia Afrin (ID:2012-1-70-047), hereby declare that the dissertation entitled **"Phytochemical and Biological Investigation of *Arachis hypogaea*"** , submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy is a genuine & authentic thesis work carried out by me during Fall 201-Spring 201 under the supervision and guidance of Dr. Repon Kumer Saha, Assistant Professor, Department of Pharmacy, East West University.

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## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## ABSTRACT

**Objective:** Groundnut [Peanut] (Arachis hypogaea L.) is one of the world's most popular oil seed crops which is grown as an annual plant but perennial growth is possible in climates which are warm until harvest. The objective of this study is pharmacological activities investigation and comparison among different varieties of ***Arachis hypogea*** available in Bangladesh.

**Methods:** Thin layer chormatogarphy were used to detect the presence of various types of compound in crude methanolic extracts derived from the ***Arachis hypogaea*** pods and shells. Crude plant powders were extracted sequentially with methanol and then undergone vacuum Liguid Chrmatograpy (VLC). Later the various fractions were screened for the presence of significant anti-diabetic, antimicrobial and anti-inflammatory pharmacological activity. Disc diffusion assay was performed to show the antibacterial effect using gram positive, gram negative strains of bacteria and fungi. In vitro antioxidant effects were measured by DPPH scavenging assay. In vitro anti- diabetic assay was carried out by Glucose uptake in Yeast cells Finally, the anti-inflammatory activities of different fraction of *Arachis hypogaea* were investigated for In-vitro anti-inflammatory activity by human red blood cell membrane stabilization (HRBC) method. Lastly, receptor binding activities were performed by hemagglutination inhibition assay.

**Result:** The different fractions of *Arachis hypogaea* have shown high antioxidant and anti diabetic activities. In addition the shell and nut lower layers of primary VLC fractions have shown relatively good anti bacterial activities, notably the against Gram negative strains. However, very few samples have exhibited anti inflammatory properties. Those worthy of mention are shell dichloromethane and methanol fractions of primary VLC fractions. In addition the nut lower layer xylene fractions have shown substantial anti inflammatory activity. Lastly, the receptor binding activity of both shell and nut lower layer primary VLC fractions have failed to show any activity.

**Conclusion:** Under phytochemical analysis, antioxidant test & Chemical screening was done. The antioxidant property found in crude methanolic extracts derived from the ***Arachis hypogaea*** shells was notable.

## RATIONALE OF THE WORK

Groundnut [Peanut] (Arachis hypogaea L.) is one of the world's most popular oil seed crops which is grown as an annual plant but perennial growth is possible in climates which are warm until harvest. It is best cultivated in well drained sandy or sandy loam soils with pH ranging from 5.5 to 6.5. Its high content of oil and protein makes it an important commodity for both human use and livestock feed. Moreover, shells are sometimes used as fuel, for the generation of electricity.

(Imam et al, 2014)

Fabaceae, also called Leguminosae, pea family of flowering plants (angiosperms), within the order Fabales. Fabaceae, which is the third largest family among the angiosperms after Orchidaceae (orchid family) and Asteraceae (aster family), consists of more than 700 genera and about 20,000 species of trees, shrubs, vines, and herbs and is worldwide in distribution. Peanut belongs to the family Fabaceae (commonly known as the bean, pea or legume family). It is an annual herbaceous plant growing 30 to 50 cm (1.0 to 1.6 ft) tall. The leaves are nyctinastic, opposite, pinnate with four leaflets (two opposite pairs; no terminal leaflet); each leaflet is 1 to 7 cm (⅜ to 2¾ in) long and 1 to 3 cm (⅜ to 1 inch) across.

*Arachis hypogea* is an annual spreading, branched herb, stem 30-80 cm long. The leaves are 8-12 cm long, imparipinnate, with leaflets of the shape oblong to obovate, 2-5 cm long. The flowers are axillary, few, fascicled, yellow, about 8 mm long. Pods are 1-5 cm long, oblong, constricted between seeds, ripening underground. Unripe fruit is lactagogue. Seed and seed oils are astringent to the bowels. The oil is aperient and emollient; used as a substitute for olive oil. The oil predominates in mono unsaturated fats, beneficial for cardiac patients, also preventive of heart attacks. Ethanolic extract of defatted peanuts shortened bleeding time; had excitatory effects on smooth muscles of experimental animals, but inhibitory effect on isolated frog heart (Asolkar et al., 1992).

The aim of this research project was to carry out the characterization of the functional molecules present in the methanolic extract of leaves of the *Arachis hypogaea* and investigate their biological activities.

# UNIT ONE- INTRODUCTION

## INTRODUCTION

Plants, also called green plants, are multicellular eukaryotes of the kingdom Plantae. They form an unranked clade Viridiplantae(Latin for green plants) that includes the flowering plants, conifers andother gymnosperms, ferns, clubmosses, hornworts, liverworts,mosses and the green algae. Green plants exclude the red and brown algae, the fungi, archaea, bacteria and animals.

Precise numbers are difficult to determine, but as of 2010, there are thought to be 300–315 thousand species of plants, of which the great majority, some 260–290 thousand, are seed plants. Green plants provide most of the world's molecular oxygen and are the basis of most of the earth's ecologies, especially on land. Plants that produce grains, fruits and vegetables form mankind's basic foodstuffs, and have been domesticated for millennia. Plants are used as ornaments and, until recently and in great variety, they have served as the source of most medicines and drugs. The scientific study of plants is known as botany, a branch of biology.

The study of plant uses by people is termed economic botany or ethnobotany; some consider economic botany to focus on modern cultivated plants, while ethnobotany focuses on indigenous plants cultivated and used by native peoples. Human cultivation of plants is part of agriculture, which is the basis of human civilization. Plant agriculture is subdivided into agronomy, horticulture and forestry.

Thousands of plant species are cultivated for aesthetic purposes as well as to provide shade, modify temperatures, reduce wind, abate noise, provide privacy, and prevent soil erosion. Plants are the basis of a multi-billion dollar per year tourism industry which includes travel to historic gardens, national parks, rainforests, forests with colorful autumn leaves, and the National Cherry Blossom Festival.

Plants are the source of many natural products such as essential oils, natural dyes, pigments, waxes, resins, tannins, alkaloids, amber and cork. Products derived from plants include soaps, shampoos, perfumes, cosmetics, paint, varnish, turpentine, rubber, latex, lubricants, linoleum,

plastics, inks, and gums. Renewable fuels from plants include firewood, peat and many other biofuels. Coal and petroleum are fossil fuels derived from the remains of plants. Olive oil has been used in lamps for centuries to provide illumination.

Plants are also a primary source of basic chemicals, both for their medicinal and physiological effects, as well as for the industrial synthesis of a vast array of organic chemicals. Medicines derived from plants include aspirin, taxol, morphine, quinine, reserpine, colchicine, digitalis and vincristine. There are hundreds of herbal supplements such as ginkgo, Echinacea, feverfew, and Saint John's wort. Pesticides derived from plants include nicotine, rotenone, strychnine and pyrethrins. Certain plants contain psychotropic chemicals which are extracted and ingested, including tobacco, cannabis (marijuana), opium, and cocaine. Poisons from plants include ricin, hemlock and curare.

In genetics, the breeding of pea plants allowed Gregor Mendel to derive the basic laws governing inheritance, and examination of chromosomes in maize allowed Barbara McClintock to demonstrate their connection to inherited traits. The plant *Arabidopsis thaliana* is used in laboratories as a model organism to understand how genes control the growth and development of plant structures. Space stations or space colonies may one day rely on plants for life support.

## HISTORY OF MEDICINAL PLANT

Medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind. Search for eternal health and longevity and to seek remedy to relieve pain and discomfort prompted the early man to explore his immediate natural surrounding and tried many plants, animal products and minerals and developed a variety of therapeutic agents. Over millenia that followed the effective agents amongst them were selected by the process of trial, error, empirical reasoning and even by experimentation. These efforts have gone in history by the name discovery of 'medicine'. In many eastern cultures such as those of India, China and the Arab/Persian world this experience was systematically recorded and incorporated into regular system of medicine that refined and developed and became a part of the Materia Medica of these countries. The ancient civilization of India, China, Greece, Arab and other countries of the world developed their systems of medicine independent of each other but all of them were predominantly plant based. But the theoretical foundation and the insights and in depth understanding on the practice of medicine that we find in Ayurveda is much superior among organized ancient systems of medicine (Rahman, 2007).

### Natural product research and drug discovery

Nature appears to be a therapeutic cornucopia to treat superfluity of diseases ranging from common cold to multifarious type of illness since the dawn of civilization. Overwhelming evidence has accumulated showing that natural products from plants, microorganisms and marine organisms comprise major portion of the total repertoire of the available therapeutic drugs. Products of natural origins are often called natural products. Natural products include: an entire organism (e.g., a plant, an animal, or a microorganism) that has not undergone any kind of processing or treatment other than a simple process of preservation (e.g., drying), part of an organism (e.g., leaves or flowers of a plant, an isolated animal organ), an extract of an organism or part of an organism, and exudates, and pure compounds (e.g., alkaloids, glycosides, sugars,

flavonoids, coumarins, lignans, steroids, terpenoids, etc.) isolated from plants, animals, or microorganisms (Samuelsson, 1999). However, in most cases the term natural products refer to secondary metabolites, small molecules (mol wt <2000 amu) produced by an organism that are not strictly necessary for the survival of the organism (Cannell, 1998).

Natural products have played a key role in drug discovery research, as many medicines are either natural products or derivatives thereof. Indeed, it is estimated that about 40% of all medicines is either natural products or their semi-synthetic derivatives. This may not be surprising as herbal medicine is a tradition of healthcare since ancient times and natural extracts screening has been one of the roots of drug discovery research, where erythromycin and rifampicin (bacterial infections), statins (hyperlipidemia), quinines and artimesinin (malaria), paclitaxel, vinblastine and vincristine (cancer), are a few well-known natural products-based medicines. For bacterial infections, over 80% of all medicines in clinical use is either natural products or their derivatives, while for anticancer agents over 60% of all drugs is either natural products or derivatives thereof; examples of several potential lead molecules are vincristine, vinblastine, taxol, camptothecin, podophyllotoxin, combretastatins, etc which have been isolated from plants for successful use in cancer treatment (Newman and Cragg, 2007). In earlier times, all drugs and medicinal agents were derived from natural substances, and most of these remedies

**The Plants Role in Human:**

A modern dictionary defines health as soundness of physical, mental or moral condition especially freedom from pain or diseases. But true health is more than that- it includes the joy of living, the power and ability to lead a satisfying and purposeful life.

Modern drugs or conventional medicine is often viewed as impersonal, emphasizing crisis intervention. It is not only expensive also many of them bring about side effect, which are sometimes more dangerous than the disease itself. Plants contain natural substances that can promote health and alleviate illness. They are source of medicines directly. The origins of medicine are tied up with plants. Alcohol was produced from starch early in civilization for use as a beverage preservative, but also as a surface sterilizing agent. Quinine from plant bark prevents malaria. Morphine, codeine, and cocaine are useful as local anesthetics and serveother

purposes through side effects too. Digitoxin from foxglove has been used to regulate heartbeat. Caffeine from plants is an important daily stimulant for many humans. Nicotine and A-9-THC are important recreational drugs. There are probably many more important medicines to be found in the tropical forests of the world where countless species remain unknown. Medicinal drugs now used in the developed world come from only about 95 of the 250,000 known species of flowering plants on earth. The study of new plants can and has led to the creation of medicines that can save lives and cure illnesses. As the tropical rainforest shrinks day by day, the potential to discover new plants shrinks as well, yet few researchers are actively seeking new plant species in the jungles of South America.

**Approaches to natural product research and drug discovery:**

Different approaches to drug discovery from plants can be enumerated as: random selection followed by chemical screening, random selection followed by one or more biological assays, follow-up of biological activity reports, follow-up of ethno medical (traditional medicine) use of plants, use of appropriate plant parts as such in powdered form or preparation of enriched / standardised extracts (herbal product development), use of a plant product, biologically potent but beset with other issues, as a lead for further chemistry, and single new compounds as drugs. The objective of the later approach is the targeted isolation of new bioactive plant products, i.e. lead substances with novel structures and novel mechanisms of action. This approach has provided a few classical examples, but the problem most often encountered here is not enough availability. The problem of availability can be overcome by semi-synthesis/synthesis or using tissue culture techniques (by genetically modifying the biosynthetic pathway of the compound of interest).

Drug discovery from plants involves a multidisciplinary approach combining botanical, ethnobotanical, phytochemical and biological techniques. The search for bioactive chemicals from the unstudied part of the plant kingdom can be conducted essentially with three methods (Cotton, 1996): the random method involves the collection of all plants found in a given area of study, phylogenetic targeting means the collection of all members of those plant families which are known to be rich in bioactive compounds, and the ethnobotanical approach is based on the traditional knowledge of medicinal plant use. It has been suggested that the ethno-directed

sampling is most likely to succeed in identifying drugs for use in the treatment of gastrointestinal, inflammatory and dermatological complaints. Strategies for research in the area of natural products have evolved quite significantly over the last couple of decades. These can be broadly divided into two categories:

**Older approach**

- Focused on chemistry of compounds from natural sources, but not on activity.
- Straightforward isolation and identification of compounds from natural sources followed by testing of biological activity in animal model.
- Chemotaxonomic investigation.
- Selection of organisms primarily based on ethnopharmacological information, folkloric reputations, or traditional uses.

**Modern approach**

- Bioassay-directed (mainly *in vitro*) isolation and identification of active lead compounds from natural sources.
- Production of natural products libraries.
- Production of active compounds by cell or tissue culture, genetic manipulation, natural combinatorial chemistry and so on.
- More focused on bioactivity.
- Introduction of the concepts of dereplication, chemical fingerprinting, and metabolomics.
- Selection of organisms based on ethno pharmacological information, folkloric reputations, or traditional uses, and also those randomly selected.

**History of Traditional Medicinal Practices in Bangladesh:**

The practice of Traditional medicine is deeply rooted in the cultural heritage of Bangladesh and constitutes an integral part of the culture of the people of this country. Different forms of Traditional medicines have been used in this country as an essential means of treatment of diseases and management of various health problems from time immemorial. The practice of traditional medicine in this country has flourished tremendously in the recent years along with that of modern medicine. As a result, even at this age of highly advanced allopathic medicine, a large majority (75-80%) of the population of this country, particularly in the rural and semi-urban areas, still prefer to use traditional medicine in the treatment of most of their diseases even though modern medical facilities may be available in the neighbourhood. However, the concept, practice, type and method of application of traditional medicine vary widely among the different ethnic groups living in different parts of the country according to their culture, living standard, economic status, religious belief and level of education. Thus traditional medicine practice in Bangladesh includes both the most primitive forms of folk medicine (based on cultural habits, superstitions, religious customs and spiritualism) as well as the highly modernized Unani and Ayurvedic systems (based on scientific knowledge and modern pharmaceutical methods and technology). These various aspects of Traditional medicine practice in Bangladesh, their current official status (acceptability, recognition, etc.) in the country as a means of treatment, and their contribution to, and impact on, the overall health management programmes of the country are described and discussed in this paper supported by documentary evidences and scientific data. **(**Ghani, 1998)

The following contains a list of crude drugs used in different disease condition in Bangladesh

| **Local Name** | **Scientific Name** | **Family** | **Usable part** | **Indication** |
|---|---|---|---|---|
| Apang | *Achyranthes paniculata* | Amaranthaceae | Whole plant | Dysentry, piles, Constipation, Arthritis, skin disease |

| | | | | |
|---|---|---|---|---|
| Kalomegh | *Andrographis pariculata* | Acanthaceae | Whole plant | Metabolic disorder, worm killer, gastric fever, dysentery, liver disease |
| Akondo | *Caltropis procera* | Asclepiadaceae | Root, leaf, bark, flower extract of leaf | Ulcer, tooth pain, chronic dysentery, cold, asthema |
| Onnontomul | *Hemidesmus indicus* | Asclepiadaceae | Root and whole plant | Appetizer, arthritis, diabetes |
| Orjun | *Terminalia arjuna* | Combrataceae | Bark | Heart disease, tuberculosis, piles, diarrhea |
| Ultokombol | *Abroma augusta* | Sterculiaceae | Root, bark and leaf | Vaginal pain, sexual disease |
| Ghritokumari | *Aloe indica* | Liliaceae | Extract of leaf | Headache, sexual disease, metabolic problem, fever |
| Thankuni | *Centella asiatica* | Apiaceae | Whole plant | Pain killer, diabetes, metabolic problem, chronic dysentery, expectorant |
| Telakucha | *Eoccinia cordifola* | Cucarbitace | Leaf and root | Diabetes, cold, ulcer, appetizer |
| Keshraj | *Eclipsta prostrata* | Asteraceae | Whole plant | Cold, hair loss treatment, headache |
| Kulakhara | *Hygrophilla* | Acanthaceac | See, leaf, stem | Liver disease, |

| | | | | |
|---|---|---|---|---|
| | *schulli* | | | ulcer,removal of liver stone, bleeding |
| Gondho vadule | *Paederia foetida* | Rubiaceae | leaf | Dysentry, cold, ulcer, arthritis, metabolic disorder |
| Dhatura | *Datura metal* | Solanaceac | Root, leaf, seed | Pain killer, worm killer, poison |
| Bohera | *Terminalia belerica* | Combretaceae | Fruit | Constipation. Diarrhea, fever, cough, piles, gastric heart disease |
| Dadmordon | *Cassia alata* | Fabaceae | Leaf | Skin disease, poison |
| Pudina | *Mentha viridis* | Labiatae | Whole plant | Metabolic disorder, gastric |
| Bashak | *Adhatoda vasica* | Acanthaceae | Leaf, root | Cough, tuberculosis, asthema, cold, blood purifier |
| Joshtimodhu | *Hydrandea arborescons* | Saxifrazaceac | Leaf, flower fruit | Liver disease, ulcer, hormonal disease, cold, throat pain |
| Shorpogondha | *Raoulfia Serpentina* | Apocynaceae | Leaf, root | Blood pressure, dysentery, diarrhea, pain killer |
| Neem | *Azadirachta indica* | Meliaceae | Root, leaf, bark | Skin disease, worm killer, arthritis, jaundice, tooth disease, antiviral |

| | | | | |
|---|---|---|---|---|
| Bel | *Aegle marmelos* | Rutaceae | Fruit | Dysentery, diarrhea |
| Anaras | *Ananas comosus* | Bromeliaceae | Fruit , leaf | Jaundice |
| Jaam | *Syzygium cumini* | Myrtaceaae | Fuit, seed | Dysentery, diarrhea |

(Sadi, 2012)

**Use of Plant Waste:**

In Egypt, there are many sources of fruit wastes but there is lake of information about their content and activity of antioxidant compounds. Recently, the use of fruit and vegetable waste to reduce environmental pollution has become a very popular alternative because these residues are important sources of polyphenols. Agricultural and industrial residues are attractive sources of natural antioxidants and dietary fiber. (Karkare, 2007)

Medicinal plants represent a rich source of antimicrobial agents. There is also an urgent need to search for a new antimicrobial compounds with novel mechanisms of action because there have been an alarming increase in the incidence of new infections diseases, as well as the development of resistance to the antibiotics in current clinical trials.(SEKAR et al, 2014)

Scientist investigates the extraction and identification of antioxidant compounds in some vegetable and fruit wastes. New by-products applications should be investigated to have a positive environmental impact or to turn them into useful products. Accordingly, the functional properties of some peel components such as, pectin, flavonoids, carotenoids, limonene and polymethoxy flavones should be considered. (Ahmed and Al-Sayeda, 2013)

**Overview of the Family Fabaceae**

**Fabaceae,** also called **Leguminosae**, pea family of flowering plants (angiosperms), within the order Fabales. Fabaceae, which is the third largest family among the angiosperms after Orchidaceae (orchid family) and Asteraceae (aster family), consists of more than 700 genera and about 20,000 species of trees, shrubs, vines, and herbs and is worldwide in distribution. The most

important commercial species include *Glycine max*(soybean), *Pisum sativum* (garden pea), *Arachis hypogaea* (peanut [groundnut]), and *Medicago sativa* (alfalfa [lucerne]). Most woody species are tropical; herbaceous (i.e., nonwoody) species occur mainly in temperate regions. (Encyclopedia Britannica, 2015)

The leaves are stipulate, nearly always alternate, and range from pinnately or palmately compound to simple. Like the other legume families the petiole base is commonly enlarged into a pulvinus. The flowers are strongly perigynous, zygomorphic, and commonly in racemes, spikes, or heads. The perianth commonly consists of a calyx and corolla of 5 segments each. The petals are overlapping (imbricate) in bud with the posterior petal (called the banner or flag) outermost (i.e., exterior) in position. The petals are basically distinct except for variable connation of the two lowermost ones called the keel petals. The lateral petals are often called the wings. The androecium most commonly consists of 10 stamens in two groups (i.e., they are diadelphous with 9 stamens in one bundle and the 10th stamen more or less distinct). The pistil is simple, comprising a single style and stigma, and a superior ovary with one locule containing 2-many marginal ovules. The fruit is usually a legume. The fruit is technically called a legume or pod. It is composed of a single seed-bearing carpel that splits open along two seams. Legume fruits come in an enormous variety of shapes and sizes, including indehiscent pods that do not split open. Of all the legumes, the peanut is especially fascinating because it develops below the ground.

Fabaceae, range in habit from giant to small annual herbs, with the majority being herbaceous perennials. Plants have indeterminate inflorescences, which are sometimes reduced to a single flower. The flowers have a short hypanthium and a single carpel with a short gynophore, and after fertilization produce fruits that are legumes. The Leguminosae have a wide variety of growth forms including trees, shrubs or herbaceous plants or even vines or lianas. The herbaceous plants can be annuals, biennials or perennials, without basal or terminal leaf aggregations. They are upright plants, epiphytesor vines. The latter support themselves by means of shoots that twist around a support or through cauline or foliar tendrils. Plants can be heliophytes, mesophytes or xerophytes.

Many species have leaves with structures that attract ants that protect the plant from herbivore insects (a form of mutualism). Extrafloral nectariesare common among the Mimosoideae and the Caesalpinioideae, and are also found in some Faboideae (e.g. *Vicia sativa*). In some *Acacia*, the modified hollow stipules are inhabited by ants and are known as domatia.

Many Fabaceae host bacteria in their roots within structures called root nodules. These bacteria, known as rhizobia, have the ability to takenitrogen gas ($N_2$) out of the air and convert it to a form of nitrogen that is usable to the host plant ( $NO_3^-$ or $NH_3$ ). This process is called nitrogen fixation. The legume, acting as a host, and rhizobia, acting as a provider of usable nitrate, form a symbiotic relationship.

Figure 1.1 *Vicia sativa*

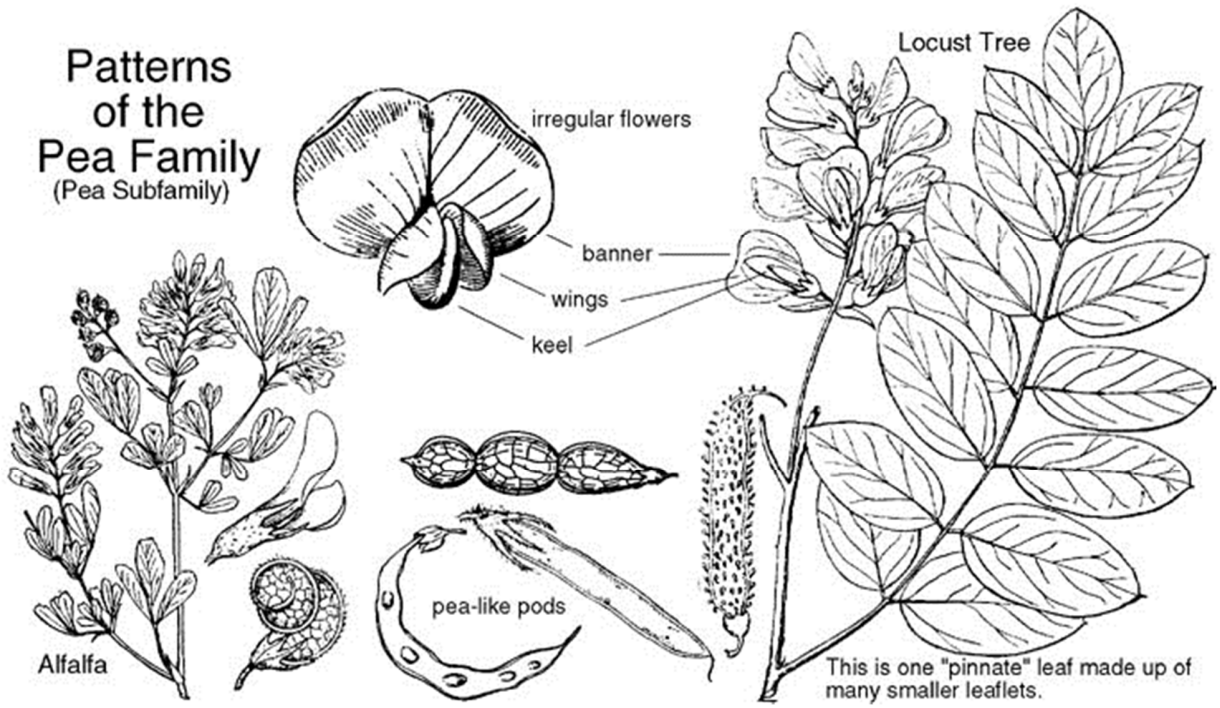



Figure 1.2 Fabaceae

**Description of the Plant**

Peanut is also known as ground nut, earth nut, pygmy nut, monkey nut or ground bean. Its scientific name is *Arachis hypogaea*. Peanuts actually grow underground, as opposed to nuts like walnuts, almonds etc. that grow on trees. Peanuts, along with beans and peas, belong to the single plant family, Leguminosae. Legumes are edible seeds enclosed in pods. It is considered as the world's fourth most important source of edible vegetable oil and third most important source of vegetable protein.

About 5000 years ago peanuts were first grew in Brazil-Bolivia-Peru region. In the 1500s century Spanish and Portuguese explorers shipped peanuts from South America to Asia, Europe and Africa. An American named George Washington Carver began his research work and developed more than 300 other uses for peanuts and improved peanut horticulture so much that he is considered by many to be the ''father of the peanut industry''.

Peanut was introduced in Bangladesh and in this region by the Portuguese sailors along with other vegetables and fruits. Nowadays peanuts are cultivated around the world almost in 80

countries. In Bangladesh peanuts are cultivated in Noakhali, Faridpur, Kishoreganj, Patuakhaliu, Rangpur and Dhaka districts in 35000 hectors of lands and about 40,000 metric tons of peanuts produced annually.

Peanut is an annual herbaceous plant growing 30 to 50 cm tall. Peanut plants grow best in sandy or loose soil with warm, sunny weather and moderate rain. Most peanuts need about five months to grow to maturity. The leaves of the peanuts plants are opposite and they grow as pairs in groups four. It is interesting that peanuts leaflets fold up in pairs at night. Each leaflet is 1 to 7 cm long and 1 to 3 cm broad. The flowers are a typical pea flower in shape, 2 to 4 cm across bright yellow with reddish veining. Peanut flower is a self-pollinating flower and grows low on the plant. Most flowers bloom for one day and then wilt. Once the flower is pollinated, the petals fall off and the ovary starts to enlarge. The budding ovary grows a small stem and the embryo penetrates the soil where the peanut starts to form into a legume pod. Pods are 3 to 7 cm long containing 1 to 4 seeds. The pods, which are ripen within 120 to 150 days after the seeds are planted.

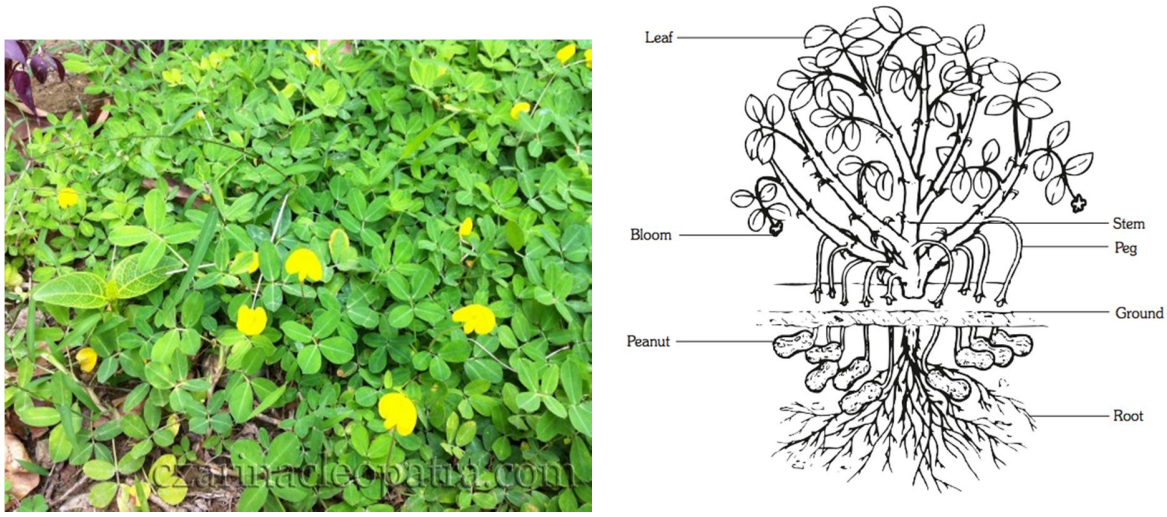



Figure 2.1 peanut plant

All parts of the peanut plant can be used. Peanuts contain 25 to 32% protein (average of 25% digestible protein) and 42 to 52% oil. . Peanuts are naturally cholesterol-free. In fact, peanuts contain over 30 essential nutrients and phytonutrients. They contain more anti oxidants than

grapes, green tea, tomatoes, spinach, broccoli and carrots. Despite of its utility some people are allergic to peanuts.



Figure2.2 Flower of peanut Figure2.3 Pods after picking

**Growth and Development:**

The optimum soil temperature for seed germination is 25–30°C. Low temperatures retard germination and development and increase the risk of seedling diseases. Upon germination, the primary root elongates rapidly, reaching 10–12 cm before lateral roots appear. As growth proceeds, the outer layer of the primary root of a seedling is sloughed off so that root hairs do not form. Branching is dimorphic, with vegetative branches and reduced reproductive branches. Secondary and tertiary vegetative branches can develop from the primary vegetative branches. Flowering may start as early as 20 days after planting, but 30–40 days after planting is more usual. The number of flowers produced per day decreases as the seeds mature. (Ingale et al,2011)

Figure 2.4 Stages of peanut development

**Harvesting:**

Groundnut seeds are often planted at a depth of 4–7 cm at a rate of 60–80 kg/ha. Groundnut pods intended for sowing are often hand-shelled 1–2 weeks before sowing. Only fully mature pods are selected. Before sowing, groundnut seed may be treated with a fungicide to control seedling diseases. In general, early sowing improves yields and seed quality. (Ingale et al,2011)

Figure 2.5 harvested peanuts

**TAXONOMY**

| | |
|---|---|
| Kingdom | Plantae – plantes, Planta, Vegetal, plants |
| Subkingdom | Viridiplantae |
| Infrakingdom | Streptophyta – land plants |
| Superdivision | Embryophyta |
| Division | Tracheophyta – vascular plants, tracheophytes |
| Subdivision | Spermatophytina – spermatophytes, seed plants |
| Class | Magnoliopsida |
| Superorder | Rosanae |
| Order | Fabales |
| Family | Fabaceae – peas, legumes |
| Genus | Arachis L. – peanut |
| Species | Arachis hypogaea L. – peanut |

(USDA PLANTS, 2010)

**Nutritional Value of peanut:**

**Table 1.1 Nutritional value of peanut**

| **Nutritional value per 100 g (3.5 oz)** | |
|---|---|
| **Energy** | 2,385 kJ (570 kcal) |
| **Carbohydrate** | 21 g |
| **Dietary fiber** | 9g |
| **Fat** | 48 g |

| | |
|---|---|
| **Saturated** | 7g |
| **Monosaturated** | 24g |
| **Polysaturated** | 16g |
| **Protein** | 25g |
| **Tryptophan** | 0.2445 g |
| **Threonine** | 0.859 g |
| **Isoleucine** | 0.882 g |
| **Leucine** | 1.627 g |
| **Lysine** | 0.901 g |
| **Methionine** | 0.308 g |
| **Cystine** | 0.322 g |
| **Phenylalanine** | 1.300 g |
| **Tyrosine** | 1.020 g |
| **Valine** | 1.052 g |
| **Arginine** | 3.001 g |
| **Histidine** | 0.634 g |
| **Alanine** | 0.997 g |
| **Serine** | 1.236 g |
| **Aspartic acid** | 3.060 g |
| **Glutamic acid** | 5.243 g |
| **Glycine** | 1.512 g |
| **Proline** | 1.107 g |
| **Vitamins** | |
| **Thiamine (B1)** | 0.6 mg (52%) |
| **Riboflavin (B2)** | 0.3 mg (25%) |

| | |
|---|---|
| **Niacin (B3)** | 12.9 mg (86%) |
| **Pantothenic acid (B5)** | 1.8 mg (36%) |
| **Vitamin B6** | 0.3 mg (23%) |
| **Folate (B9)** | 246 µg (62%) |
| **Vitamin C** | 0.0 mg (0%) |
| **Vitamin E** | 6.6mg (44%) |
| **Magnesium** | 184 mg (52%) |
| **Iron** | 2 mg (15%) |
| **Calcium** | 62 mg (6%) |
| **Zinc** | 3.3 mg (35%) |
| **Potassium** | 332 mg (7%) |
| **Phosphorus** | 336 mg (48%) |
| **Manganese** | 2.0 mg (95%) |
| **Water** | 4.26g |

(USDA, 2015)

**Uses of Peanut:**

- In India seeds are used for respiratory problems and the husks of pods are boiled and the liquid is used to treat hypertension
- They are rich in resavtrol that have been found to control cholesterol and circulatory diseases. Furthermore the red skins contain component called oligomeric procyanidins. This has proven to have anti-arteriosclerotic properties and helps prevent endometriosis.

- Togather, resavtrol and  oligomeric procyanidins helps fight cancer
- It contains phytoestrogen genisteins that help prevent hot flushes or depression related to PMS. Along with helping to prevent the formation of cancer cells, it also prevents vision loss and macular degeneration in diabetic patients.
- Peanuts possess galactogouge properties.
- Peanuts boost the immune system and prevent lymphatic disorders
- Peanut oil obtained from cold pressing has medicinal uses

(Botanical, 2015)

**Chemical Constituents:**

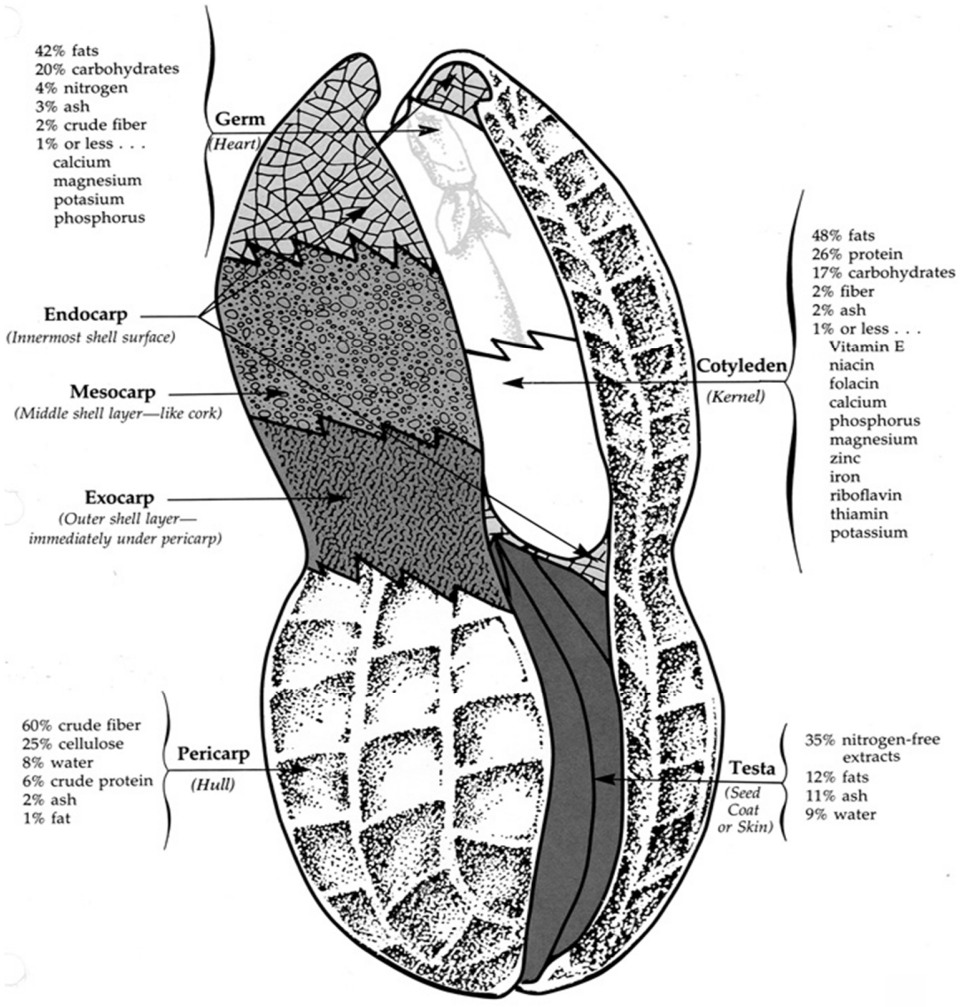


Figure 3.1 Components and Composition of peanut

Chemical components of peanut-

- Acids- Arachidic Acids, aspartic acid, behenic acid, cholorogenic acid, stearic acid, gadoleic acid, gentisic acid, lauric acid, linoleic acid, oleic acid, p-coumaric acid, palmitic, palmitoleic, ascorbic acid
- Arachin
- Lecithin
- Flavonoid- quercetin
- Amino acids- aspartic acid, glutamic acid, alanine, arginine, cysteine, phaenylalanine, methionine, proline, serine, tyrosine, glucine ,lycine, leucine, isoleucine, histidine, threonine, tryptophan, valine
- Minerals- aluminium, sulfur, cadmium, zinc, copper, boron, copper, iron, selenium, sodium, calcium, magnesium, phosphorus, potassium
- Fat
- Carbohydrates, cellulose
- Vitamins- niacin, folacin, riboflavin, thiamin

(Botanical, 2015)

Chemical components of peanut shell-

Poteins- 8.2%

Cellulose- 37%

Carbohydrates- 2.5%

Hemicelluloses- 18.7%

Lignin- 28%

(Jaishankar et al, 2014)

**Related Chemical Structures:**

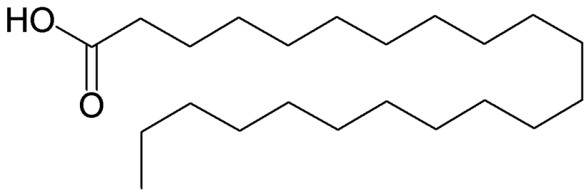



Figure: Behenic acid

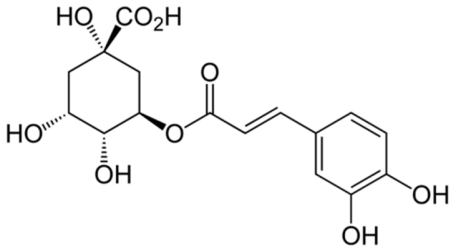



Figure: Cholorogenic acid

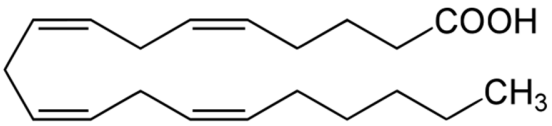



Figure: Arachidonic acid

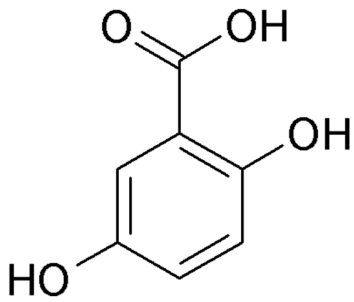



Figure: Gentisic acid

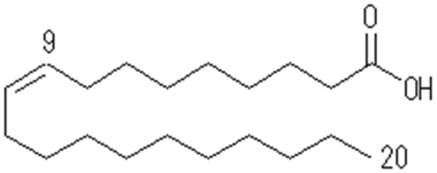



Figure: Gadoleic acid

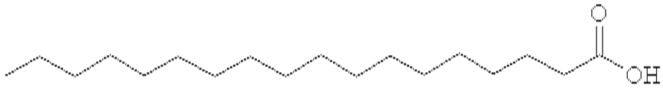



Figure: Stearic Acid

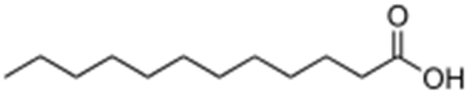



Figure: Lauric acid

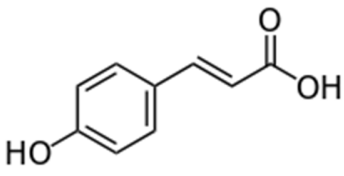



Figure: p-coumaric acid

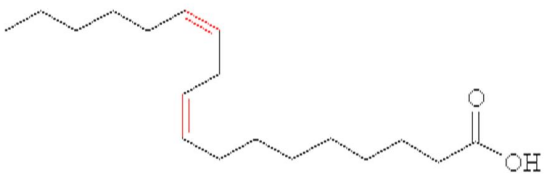



Figure: Linoleic acid

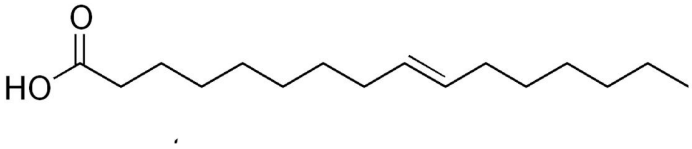



Figure: Palmitoleic acid

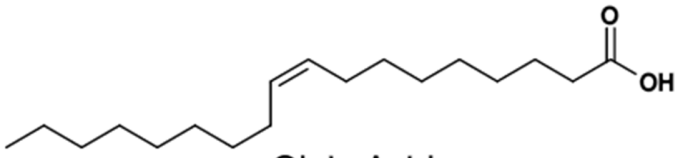



Figure: Oleic acid

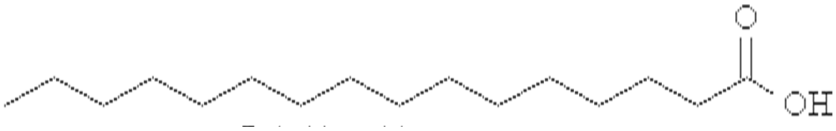



Figure: Palmitic acid

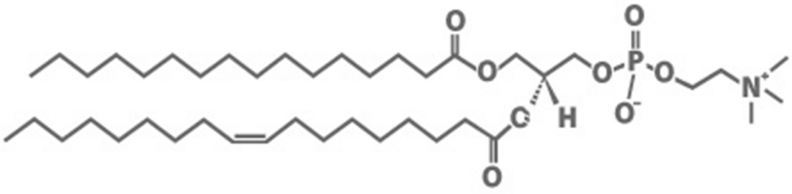



Figure: Lecithin

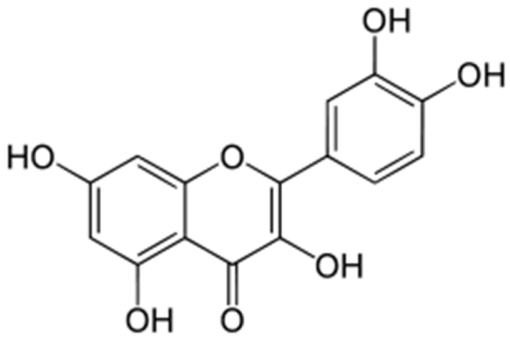



Figure: Quercetin

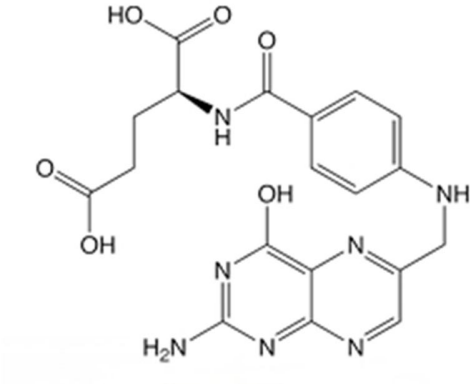



Figure: Folacin

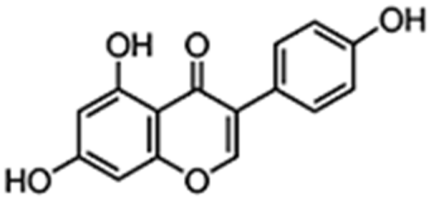



Figure: Genistein

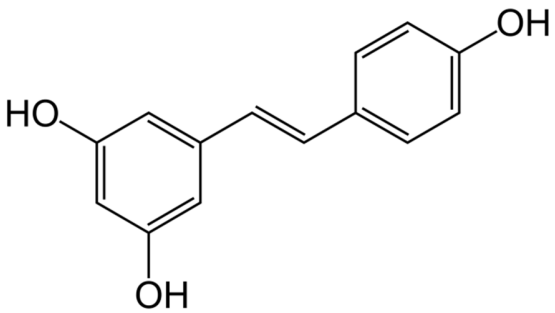
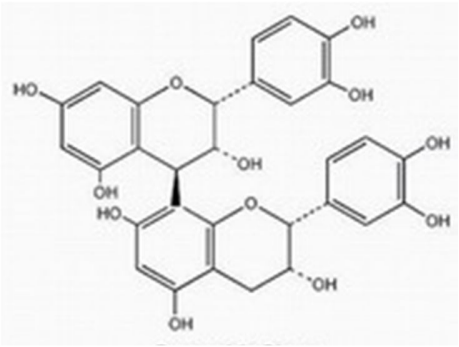



Figure: Reserveratrol



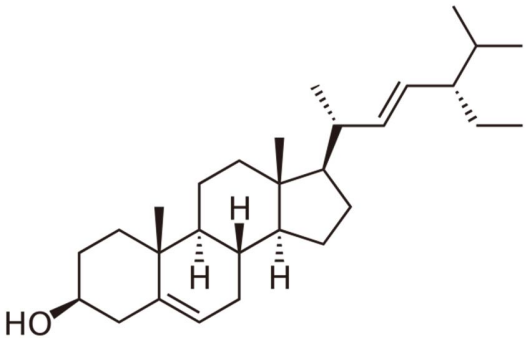



Figure: Oligomeric procyanidins

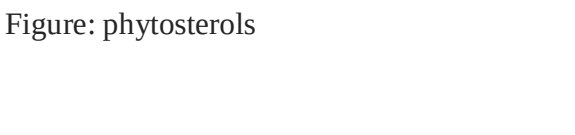


Figure: phytosterols

# UNIT TWO LITERATURE REVIEW

# LITERATURE REVIEW

## Nutritional value of seeds

The result showed that the groundnut seed contain:

- Moisture (5.529%),
- Crude fibre (1.149%)
- Lipid (46.224%)
- Crude protein (25.20%)
- Carbohydrate (21.26%)
- Ash (2.577%)
- The very high oil content of peanuts (35 to 40%)
- Calcium (0.087%)
- Phosphorus (0.29%)
- Energy (601.856%).
- Fatty acid(saturated and unsaturated) 10.44 and 33.51%

(Batal et al, 2005)

## Composition of peanut shells

Peanut shells were selected on the basis of its freshness, dryness and hard structure. It contains:

8.2% of protein, 28.8% of lignin, 37.0% of cellulose, 18.7% of hemicelluloses and 2.5% of carbohydrates.

## Physical Properties of shells

I. Its bulk density was 120-140 $kg/m^3$
II. Ash melting point (K)>1200. ( Monisha et al, 2014)

## Protein Solubility

The protein solubility at different pH ranging from 0.5 to 13.5, the maximum seeds proteins were extracted at pH 12. The serine has not been reported in the seed protein and the seed was found to contain highest amount of proline (6.412%).

**Anti-nutritional analysis**

The anti-nutritional analysis shows that cyanide content 4.818 HCN/100 g, tannin 0.412/100 g, oxalate 0.180/100g. **(**Ingale S.,Shrivatava S.K., 2011)

**Groundnut responses on growth to seed irradiation & fertilizer**

- ✓ Groundnut seeds are mainly comprised of protein, fat and carbohydrate and that is what make it sensitive to radiation induced stress, but unlike the suppressive effect high gamma rays (0.7- 2.3 KGy) have on germination and growth parameters of groundnut low doses have been reported to be beneficial stated that gamma rays produce radicals that can damage and affect differentially plant morphology, anatomy, biochemistry, and physiology depending on the irradiation level. They added that lower exposures to gamma rays were sometimes stimulatory and that several studies reported improvement of agronomic characteristics by using gamma radiation. (Imam et al, 2014)
- ✓ Increased pod yield and root- shoot ratio as a result of N addition. Nitrogen is known to be an important determinant of plant growth and development, and this might explain the increased yield in response to increased Nitrogen application. (Imam et al, 2014)

**Adsorptive activity of shells**

Making use of peanut shells as bio-adsorbents is an effective method to adsorb toxic heavy metals from effluents not polluting the ground water and at the same time utilizing the discarded open agricultural wastes in the environment for a useful purpose of waste water treatment.( Monisha et al,2014)

**Pharmacological Activity**

Receptor Binding Activity

- ✓ hemagglutinin activity for goat blood group is 1:8
- ✓ No haemagglutinin activity for chicken and human blood group,
- ✓ No trypsin inhibition was found. **(**Ingale S.,Shrivatava S.K., 201)

**Epidemiological Studies on Nut Consumption & Reduction of heart disease, Type-2 diabetes and Inflammation.**

Epidemiologic studies have associated nut consumption with a reduced incidence of coronary heart disease and gallstones in both genders and diabetes in women. Limited evidence also suggests beneficial effects on hypertension, cancer, and inflammation. Interventional studies consistently show that nut intake has a cholesterol-lowering effect, even in the context of healthy diets, and there is emerging evidence of beneficial effects on oxidative stress, inflammation, and vascular reactivity. Blood pressure, visceral adiposity and the metabolic syndrome also appear to be positively influenced by nut consumption. Thus it is clear that nuts have a beneficial impact on many cardiovascular risk factors. Contrary to expectations, epidemiologic studies and clinical trials suggest that regular nut consumption is unlikely to contribute to obesity and may even help in weight loss.

US Food and Drug Administration issued a health claim for nuts because of the link between nut consumption and a reduced risk of both CHD and intermediate biomarkers, such as blood cholesterol. The scientific evidence behind the proposal of nuts as cardio-protective foods stem from both epidemiological observations suggesting a consistent inverse association between the frequency of nut intake and development of CHD and numerous short-term clinical trials showing beneficial effects of nut intake on the lipid profile and other intermediate markers of CHD. A study of 987 diabetic women from the prospective Nurses' Health Study showed a direct association between nut consumption and increased plasma levels of adiponectin, an adipose tissue-secreted cytokine with anti-inflammatory and ant atherosclerotic properties. The third study was carried out in 772 older subjects at high risk for CHD living in Spain Nutrients 2010, 2 659 for the purpose of assessing adherence to the Mediterranean dietary pattern and its food components in relation to levels of soluble inflammatory markers. Adjusted mean serum levels of intercellular adhesion molecule-1 (ICAM-1), but not those of CRP or IL-6, decreased across increasing tertiles of nut consumption.

There were a limited number of hemorrhagic strokes in the highest categories of nut consumption, thus further studies are clearly warranted to confirm or discard this improbable adverse effect of nuts.

There was an inverse relationship between nut intake and hypertension in lean subjects but not in those who were overweight or obese at baseline.

Nut consumption was inversely associated with risk of type-2 diabetes after multivariate adjustment for traditional risk factors, with relative risks across categories of nut consumption for a 28 g serving of 1.0, 0.92, 0.84 and 0.73. Considering only lean women, a 45% risk reduction was observed in those consuming nuts five times or more per week. Consumption of peanut butter was also inversely associated with type-2 diabetes with an adjusted relative risk of 0.79 in women consuming peanut butter more than four times a week compared with those who never or almost never ate peanut butter.

**Health Benefits of Nut Consumption**

Nuts are also rich sources of other bioactive macronutrients that have the potential to beneficially affect metabolic and cardiovascular outcomes. They are an excellent source of protein (approximately 25% of energy) and often have a high content of L-arginine . As this amino acid is the precursor of the endogenous vasodilator, nitric oxide (NO), nut intake might help improve vascular reactivity. Nuts contain sizeable amounts of folate, a B-vitamin necessary for normal cellular function that plays an important role in detoxifying homocysteine Nuts are also rich sources of antioxidant vitamins (e.g., tocopherols) and phenolic compounds, also bioavailable after consumption and capable of providing a significant antioxidant load. Almonds in particular are especially rich in α-tocopherol. Nuts are cholesterol-free, but their fatty fraction contains sizeable amounts of chemically related noncholesterol sterols belonging to a heterogeneous group of compounds known as plant sterols or phytosterols. Phytosterols interfere with cholesterol absorption and thus help lower blood cholesterol when present in sufficient amounts in the intestinal lumen.

(Ross, 2010)

**Antioxidant capacity, nutritional and phytochemical content of peanut shells and roots**

Shell and roots of peanut had a significant amount of total dietary fiber, protein and ash content. Therefore, they can be utilized as feed or prebiotic compound. These peanut by-products also showed levels of total saponins and phytic acid below the ant nutritional factor threshold.Peanut roots had the highest phenolic, saponins and alkaloid contents. Positive correlations between these phytochemical contents and DPPH radical scavenging activity were found for peanut shells and peanut roots.

i) Phenolic compounds that possess redox properties which lead to the antioxidative capacities (Siddhuraju and Becker, 2003) may be present in peanut shells and roots.
ii) Specific alkaloid classes such as quinolone alkaloid with antioxidant activity (Chung and Woo, 2001) may be present in peanut shells and roots.
iii) Saponins that act as chelators of transition metals (Cu2+ or Fe2+) and results in diminished cellular sensitivity to oxidant damage (Amzal et al., 2008) may be present in peanut shells and roots.

( Sim et al, 2010)

**Chemical Characteristics (acid Value And Iodine Value) Of Peanut Oil**

The high iodine value denotes high degree of unsaturation of the oil caused by the extent of oxidation and degree of heat treatment during oil processing (Kirk and Sawyer, 1991). Peanut oil has a high smoke point relative to many other cooking oils, so is commonly used for frying foods. Its major component fatty acids are oleic acid (46.8% as olein), linoleic acid (33.4% as linolein), and palmitic acid (10.0% as palmitin)

(Mandloi et al, 2014)

**Anti inflammatory and Antioxidant Activity of Peanut skin**

The in vitro antioxidant activity of peanut skin extracts (PSE) has been reported but the associated anti-inflammatory properties have not been widely examined. This study investigated the anti-inflammatory effects of PSE on the pro-inflammatory enzyme, Cyclooxygenase-2

(COX-2) protein expression, on its downstream product, prostaglandin E2 (PGE2), and on nitrous oxide (NO) levels. Acetone peanut skin extracts and ethanol peanut skin extracts suggesting that A-PSE and E-PSE not also possess similar antioxidant properties, but also exhibit similar anti-inflammatory effects. (Lewis et al, 2013)

**Anti cancer properties of peanut plant**

When infected by a microbial pathogen, the peanut plant (Arachis hypogaea) becomes a potent producer of a distinctive set of stilbene-derived phytoalexins.3-5 Peanut stilbenoids have been considered the major sustaining factor of the plant's resistance to diseases. In addition, the health benefits of resveratrol (24) (Figure 1) from peanuts.

Anticancer properties of selected peanut stilbenoids, arachidin-1, arachidin-3, trans-30 -isopentadienyl-3, 5, and 40 -trihydroxystilbene, and resveratrol (24) were investigated. Arachidin-1 (1) appreciably induced mitochondrionmediated apoptosis at low concentrations and was demonstrated to be an effective anticancer agent that was capable of inducing caspase-independent death of cancer cells with mutations in apoptotic genes. (Sobolev et al, 2011)

Peanuts play a crucial role in weight management. Epidemiological studies have provided useful information on beneficial effects of nuts which also includes peanuts. Even though peanuts are rich in fats, they can still be included in diet for weight loss. Metal ions which are cofactors for many enzymes, vitamins that are not synthesized in the body, poly unsaturated and monounsaturated fatty acids, whose consumption can increase the levels of HDL cholesterol which is good for the heart, are present in peanuts. A diet including peanuts could provide all these vital nutrients, and play a critical role in preventing disease and promoting good health. (Settaluri et al, 2012)

# UNIT THREE: PLANT COLLECTION & IDENTIFICATION

## PLANT COLLECTION & IDENTIFICATION

Plant Collection And Identification

The nuts were collected from Savar, Dhaka, Bangladesh during April, 2015 and identified by the taxonomist of the Bangladesh National Herbarium, Mirpur, and Dhaka. A voucher specimen of the plant *Arachis hypogaea* was deposited (Accession No.: 42269) in the herbarium for further reference.

Drying Of Plant Sample

After the collection of sample it needs to be dried to make the sample extract. In general the plant material should be dried at temperature below 30 degree C to avoid the decomposition of thermo labile compounds. So sun drying can be very effective but drawback is sometimes water molecules are absorbed by the sample and hence fungus growth can affect the phytochemical study. The seeds along with the test a were dried in the sun light thus chemical decomposition does not take place.

Grinding Of Dried Sample

Small amount of plant material can be milled using grinder or blender. But if the sample is in high amount then it is easier to get powdered sample by grinding from a spice mill. Grinding improves the efficiency of extraction by increasing surface area. It also decreases the amount of solvent required for the extraction. The dried samples were ground to coarse powder with a mechanical grinder (Blender) and powdered samples were kept in clean closed containers pending extraction. During grinding of samples, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other foreign matter deposited on the grinder.

### ℘ Maceration Of Dried Powdered Sample

**Principle**

The choice of extraction procedure depends on the nature of the plant material and the components to be isolated. The principle of solid-liquid extraction is that when a solid material comes in contact with a solvent, the soluble components in the solid material move to the

solvent. Thus, solvent extraction of plant material results in the mass transfer of soluble active principle (medicinal ingredient) to the solvent, and this takes place in a concentration gradient. The rate of mass transfer decreases as the concentration of active principle in the solvent increases, until equilibrium is reached and the concentration of active principle in the solid material and the solvent are the same. Thereafter, there will no longer be a mass transfer of the active principle from plant material to the solvent. Since mass transfer of the active principle also depends on its solubility in the solvent, heating the solvent can enhances the mass transfer. Moreover, if the solvent in equilibrium with the plant material is replaced with fresh solvent, the concentration gradient is changed.

Figure 4.1 Maceration of dried peanut shells

**Procedure**

After getting the sample as dried powdered, the sample (500 Gram) was then soaked in 1000 ml of methanol for 5 days, the process is known as maceration technique. A glass made jar with plastic cover was taken and washed thoroughly with ethanol and dried. Then the dried powder sample was taken in the jar. After that methanol (1000 ml) was poured into the jar up to 1-inch height above the sample surface as it can sufficiently cover the sample surface. The plastic cover with aluminum foil was closed properly to resist the entrance of air into the jar. This process was performed for 5 days. The jar was shaken in several times during the process to get better extraction.

### Filtration Of The Extract

After the extraction process the plant extracts was filtered with sterilized cotton filter. The cotton was rinsed with ethanol and fitted in a funnel. The filtrate was collected in a beaker. Then again it was filtered and this time What man's filter was used for getting more clear extract which would be useful making the sample more concentrate in Rotary Evaporation Technique. Then the filtrate was taken into a volumetric flask and covered with aluminum foil paper and was prepared for rotary evaporation.

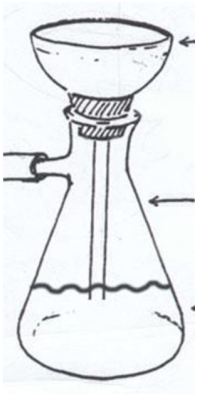


Figure 4.2: Filtration

### Sample Concentration By Rotary Evaporation Technique

**Principle**

- A rotary evaporator is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation. Rotary evaporators are also used in molecular cooking for the preparation of distillates and extracts. A rotary evaporator consists of following parts-

✓ A motor unit that rotates the evaporation flask or vial containing the user's sample.

✓ A vapor duct that is the axis for sample rotation, and is a vacuum-tight conduit for the vapor being drawn off of the sample.

✓ A vacuum system, to substantially reduce the pressure within the evaporator system.

✓ A heated fluid bath (generally water) to heat the sample.

- ✓ A condenser with either a coil passing coolant, or a "cold finger" into which coolant mixtures such as dry ice and acetone are placed.
- ✓ A condensate-collecting flask at the bottom of the condenser, to catch the distilling solvent after it re-condenses.
- ✓ A mechanical or motorized mechanism to quickly lift the evaporation flask from the heating bath.

Figure 4.3 Rotary evaporator

Affecting Factors

There are factors, omission of any one of the following factor may interfere the sample concentration procedure and thus which may interfere the phytochemical investigation.

- Remove the flask from the heat bath.

- Opening the stopcock.
- Heating the rotor.
- Turning off the vacuum/aspirator.
- Disconnecting the flask.
- Dropping flask in heat bath.

**Procedure**

After the filtration process two parts were obtained namely 'residue part' and 'filtered part or filtrate. The filtrate part, which contains the substance soluble in methanol, was putted into a 1000 ml round bottom flask (BOROSOL) and then the flask was placed in a rotary evaporator. The evaporation was done at 45 degree Celsius temperature. The number of rotation per minute was selected as 130 RPM. The pressure of the vacuum pumper machine (Biometra) was 6 bar. The water flow through the distillation chamber was also provided in a satisfactory flow rate. When the evaporation seemed to be satisfactory, then the methanolic extract was collected in a 100 mi beaker. The evaporator flask was rinsed by diethyl ether. Then the beaker was covered with aluminum foil paper and kept on the water bath for 60 minutes and the temperature of water bath maintained as 50' C. Finally the concentrated methanolic extract was found and stored in the laboratory refrigerator from which the extract was used for many chemical investigations.

## ❖ SAMPLE CONCENTRATION BY VACUUM LIQUID CHROMATOGRAPHY (VLC) TECHNIQUE

**Principle**

Chromatographic purification is an integrated part of organic synthesis. The Dry Column Vacuum Chromatography presented here, has excellent resolving power, is easily applied to large scale chromatography (up to 100 g) and is fast. Furthermore, the technique is economical and environmentally friendly due to significant reductions in solvent and the amount of silica used. Therefore, it is an excellent alternative to the commonly used Flash Column Chromatography for purification in organic synthesis.

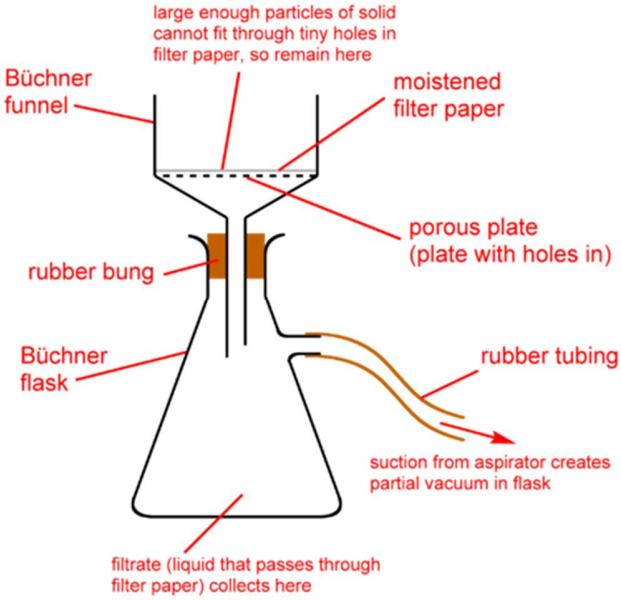



Figure 4.4 Vacuum Liquid Chromatography

**Apparatus**

- VLC chamber.
- Filter paper

**Reagents**

- Silica gel
- Hexane methanol
- Cyclohexane
- Chloroform
- Dichloromethane.
- N-butanol
- Ethanol

**Procedure**

500gm Methanol extract of *Arachis hypogaea* was further exploited in an attempt to isolate the active principle which exhibited the antibacterial activity. In the isolation procedure, different fractions were obtained by using vacuum liquid chromatography apparatus **.** A sintered glass Buckner funnel attached to a vacuum line was packed with TLC grade silica gel. The silica gel was compressed under vacuum in order to achieve a uniform layer in order to get a better separation. The methanol extract was added to an amount (200 mg) of silica gel in order to make a smooth paste. Pet ether, cyclohexane, xylene, dichloromethane, chloroform and methanol were used as mobile phase in different ratios of increasing polarity from petether to methanol. The mixture was separated according to the polarity of solvents. Each fraction was collected in a separate 100ml beakers. The fractions were monitored by thin layer chromatography. The most active fractions having the similar thin layer chromatography profile were pooled together.

Equipments and other necessary tools

During the extraction procedure and for various phytochemical tests many equipments and materials were used. Some of them are TLC plate, TLC tank, scale, pencil, TLC plate cutter, capillary tube, mortar and pestle, laminar air flow cabinet, loop, burner, micropipette tip, Petri dishes, glass rod, cotton, filter paper, funnel, hot plate, centrifugal machine, autoclave, glassware washers, stirrer, UV spectroscopy, knife, ephedrine tube, Whatman's filter paper, paper disc, incubator, vortex machine, PH meter. analytical balance, beaker (in various size), pipette, micro-pipette, rotary evaporator, hot air oven, dryer, storage cabinet, spatula, test tube, volumetric flask, conical flask, test tube holder, test tube rack, aluminum foil paper, scotch tape, refrigerator, water bath, electronic shaker, ultra violate lamp, mask, gloves, lab coat, sprayer, reagent bottle.

Chemicals and other reagents

Ferric chloride, Sodium carbonate, deionized water, Gallic acid, Sodium nitrite, Aluminum chloride, Sodium hydroxide, Hydrogen peroxide, Normal saline, Wagner's reagent, Hydrochloric acid, Glacial acetic acid, Ammonia, Phoshomolybdic acid, Acetic anhydride, Alcoholic ferric chloride, 5-aqua copper sulphate, Sodium potassium tartrate, DPPH (2,2-diphenyl-1-picrylhydrazyl), Sulfuric acid, Folin reagent, Ciocalteu reagent, prolein amino acid (protein), 1-butanol, glacial acetic acid, Ninhydrine solution, Glucose, Galactose, Maltose, Lactose, Acetone,

Phosphate buffer, Anisaldehyde, L-Ascorbic acid, potassium ferricyanide, Ttrichloro acetic acid (TCA)

Solvents for experiments

Dichloromethane, Benzene, Ammonium hydroxide, Formic acid, Dimethylsulfoxide (DMSO), Acetone, Chloroform, Distilled water, Ethanol, Methanol, Diethyl ether, Acetic acid, n-Hexane, Ethyl acetate **.**

## ☙ Lectin Extract Preparation

**Equipments and other necessary tools**

- Centrifuge machine.
- Filter paper.

**Salt and solution**

- PBS solution(pH -7.4)
- Ammonium sulphate.

**Procedure**

**1.** Fruits of *Arachis hypogaea* were collected from Savar, Dhaka.

**2.** The fruits were shade dried, seeds were removed and fruits were ground mechanically.

**3.** One hundred grams of powder was extracted overnight with 700 ml of PBS, pH 7.4, at 4°C.

**4.** The suspension was centrifuged at 12,000 g for 30 min.

**5.** The clear supernatant (crude extract) was subjected to 60% ammonium sulphate fractionation and the protein pellets were collected by centrifugation as described above.

**6.** The pellet was re-suspended in PBS, pH 7.4 and dialyzed exhaustively against the same buffer for a period of 48 h.

**7.** The resulting suspension was centrifuged at 12,000 g for 10 min and the supernatant was used for further analysis. (Hou Y. et. al., 2010)

# UNIT FOUR: METHOD & MATERIAL

## METHOD AND MATERIAL:

### A. Thin Layer Chromatography:

Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound. TLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation .TLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC is to obtain well defined, well separated spots.

### Principle of Thin Layer Chromatography:

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.

As the solvent moves past the spot that was applied, equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualization is straightforward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates. (The plate itself contains a fluorescent dye which glows everywhere except where an organic compound is on the plate). (Orgchem.colorado.edu, 2015)

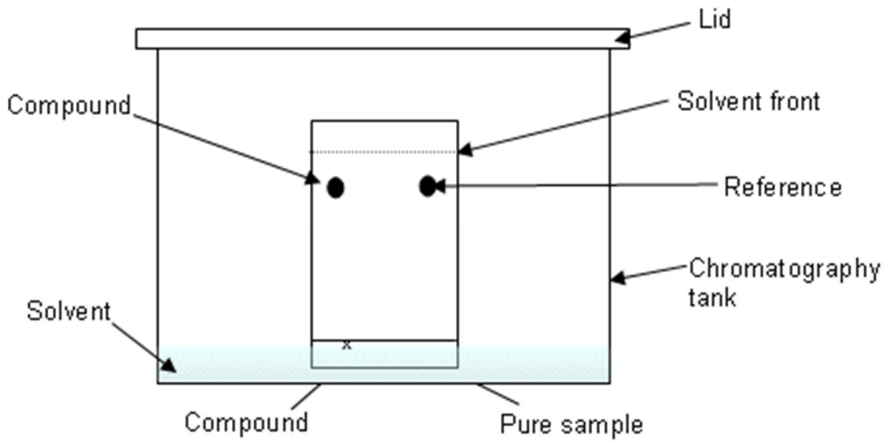



Figure 4.5 Thin layer chromatography instruments

**Solvent Systems of Thin Layer Chromatography**

Determining the optimum solvent mixture for your TLC experiment can be challenging, as there are no steadfast rules governing this procedure. It is almost entirely a matter of building experience through trial and error. However, understanding how chromatography works can make your guesswork a bit more educated.

While we have discussed a number of different interactions that occur between the mobile phase, the stationary phase, and the accompanying analyte, the factor that is most important here is polarity. The analyte exists in equilibrium between the stationary and mobile phases. If there exists a greater polar attraction between the solvent and the analyte than the silica and the analyte, then the analyte will spend more time traveling in the mobile phase along the plate than it will being attached to the stationary phase. The greater the polarity of a solvent, the faster the elution. However, this is all relative to the polarity of the analyte itself. If the analyte has a greater polarity than the solvent, it will remain more easily attached to the silica. Thus, different analytes behave differently to the polarity of a solvent, depending on their own polarities. By experimenting with solvent mixtures and keeping in mind this idea of polarity, one can achieve a desirable separation. (Umich.edu,n.d.)

- **Solvent Systems of TLC**

**Nonpolar basic solvent**

- Benzene 9 ml
- Ethanol 1 ml
- Ammonium hydroxide 0.1 ml

**Intermediate polar solvent**

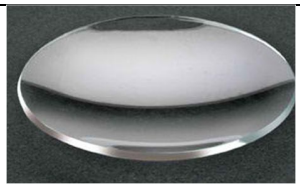
- Chloroform 5 ml
- Ethyl acetate 4 ml
- Acetic acid 1 ml

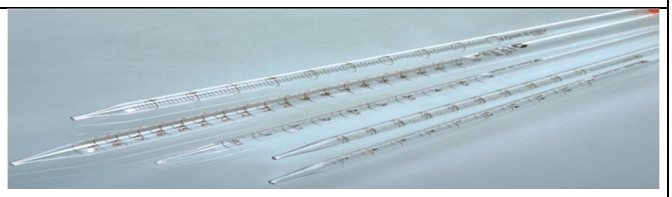
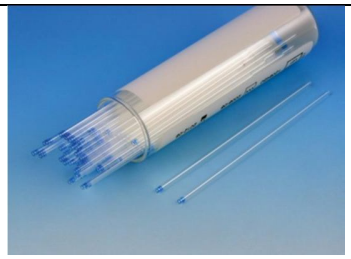
**Polar Basic solvent**

- Ethyl acetate 8 ml
- Ethanol 1.2 ml
- Water 0.8 ml

**Apparatus used for Thin Layer Chromatography**

| SL. | Apparatus | Image |
|---|---|---|

| | | |
|---|---|---|
| 1 | TLC tank |  |
| 2 | Watch glass |  |
| 3 | TLC plate |  |
| 4 | Spray bottle |  |
| 5 | Pencil |  |
| 6 | Hot plate |  |

| | | |
|---|---|---|
| 7 | Scale |  |
| 8 | UV lamp |  |
| 9 | Pipette |  |
| 10 | Capillary tube |  |
| 11 | Pumper |  |
| 12 | Tweezers |  |

| | | |
|---|---|---|
| 13 | TLC plate cutter |  |

**Chemicals Needed for Thin Layer Chromatography:**

List of chemicals for TLC

- TLC solvent:

| **Non-polar Solvent** | | **Intermediate polar Basic Solvent** | | **Polar Basic Solvent** |
|---|---|---|---|---|
| Benzene 9 ml | | Chloroform 5ml | | Ethyl acetate 8ml |
| Ethanol 1 ml | | Ethyl acetate 4ml | | Ethanol 1.2ml |
| Ammonium hydroxide 0.1 ml | | Acetic acid 1ml | | Water 0.8ml |

**Procedure for Thin Layer Chromatography:**

1. A spatula tip amount of extracted sample were taken in different watch glass and dissolved in their respective solvent, at last labeled properly.

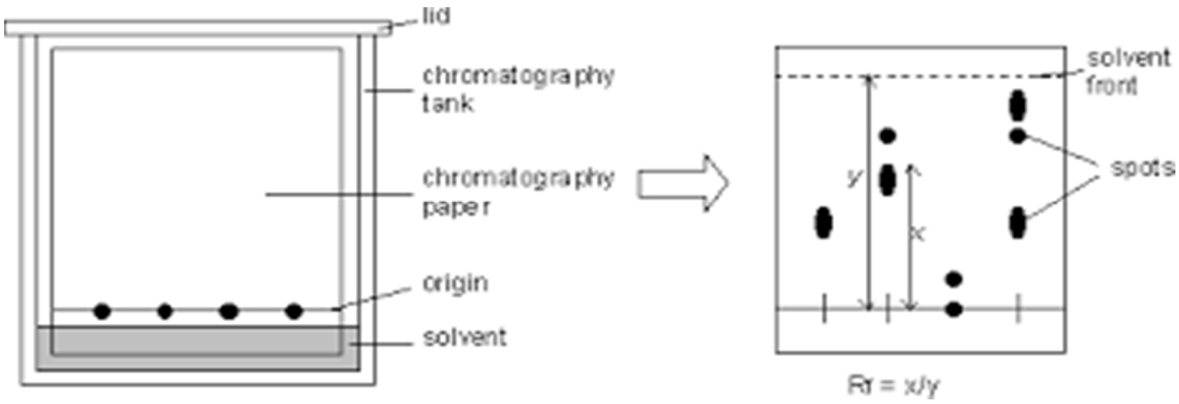
2. TLC plates were taken. The plates were marked as plate-e1, plate-2. A pencil was used to lightly mark a straight line about 0.5 cm from both end of the plates.

3. With a capillary micropipette a small spot of the extracted sample was spotted on the plates. Separate capillary micropipette was used for each sample. To avoid confusion, the spots were lightly labeled below the pencil line.

4. The TLC plates were developed by placing them in the TLC chamber that has been filled with the developing solvent. The solvent was allowed to migrate up the TLC plates until it reached the marked top end line.

5. The plates were then removed immediately and the solvent was allowed to evaporate. After that it was visualized under UV light and all the spots were marked.

6. The distance that the solvent moved was measured for the distances of all spots.




X =distance travels by mobile phase Y= distance travels by spot

Figure 4.6 Distance of spot for Rf value

7. The Rf value was then calculated. It can be calculated as:

$$Rf = \frac{\text{distance moved by the compound}}{\text{distance moved by the solvent}}$$

**Charring with $H_2SO_4$:**

Then charring of 2 plates with 10% $H_2SO_4$ solution was carried out for further confirmation.

**Staining with DPPH (2, 2-diphenyl-1-picrylhydrazyl):**

0.4% DPPH solution was prepared with methanol as solvent and labeled as stock solution. From the stock solution 10% DPPH solution was taken in a petri dish and the TLC plates were dipped

in it. The plate was visually observed for a color change after sometime. All the processes were carried out in dark place. The antioxidant active regions became yellow in color.

**Staining with FC (Folin-Ciocalteu) reagent:**

Staining with FC reagent was carried out as the same procedure above with plates. 10% FC solution was prepared with water as the solvent. The antioxidant active regions became yellow/ white in color.

## B. THIN LAYER CHROMATOGRAPHY (TLC) OF LECTIN EXTRACT

**Principle**

Thin layer chromatography is used to separate mixtures of compounds. It gives qualitative idea about the components that are present in a mixture. The experiment is usually conducted on a sheet of aluminum foil that has been coated with silica. This acts as the stationery phase for the mixture. The solvent or solvent system that runs on the stationery phase by capillary action and conducts the separation, this is known as the mobile phase. Once the sample has been spotted on, the plate and the mobile phase run, different components of the mixture separate differently due to their relative affinities for the stationery and mobile phases. Heavier components or the ones more attracted to the stationery phase remain at the bottom while components that are light and more soluble in the mobile phase travel up with it. The relative separation of the components can be studied by calculating the Retardation Factor (Rf), which is the ratio of the distance of migration of a particular substance to the distance of migration of the solvent front.

**Materials Required**

- Silica coated TLC plate.
- TLC tank.
- Spotting capillary tubes.
- Tweezers.
- Pipette.
- Pipette filter.
- Test tubes.
- Solvents.

- UV lamp

**Reagents**

- Water
- 1-butanol
- Glacial acetic acid

Table 2.1 Composition of various solvent systems for TLC for Lectin extract

| Protein test | Concentration |
|---|---|
| 1-butanol | 6ml |
| Glacial acetic acid | 2ml |
| Water | 2ml |

| Carbohydrate test | Concentration |
|---|---|
| 1-butanol | 4ml |
| Acetone | 5ml |
| $Po_4$ buffer | 1ml |

**Procedure**

- Using a pencil the baseline and the solvent front line was drawn on the TLC plate and the plate was labeled for the individual spots.
- The Lectin extract was spotted on TLC plate and the plate was dried completely in the air.

- In a TLC tank the solvent system was added. A strip of filter paper was inserted into the tank so that its bottom touched the solvent. The lid of the tank was closed and left to rest for a few minutes so that the solvent system could travel up the filter paper and saturate the chamber.
- Using a pair of tweezers the TLC plates were placed in the chamber carefully so that the baseline did not touch the solvent.
- The plate was left in the tank so that the solvent system could run up the plate by capillary action and develop the spots.
- The plate was removed from the tank using a pair of tweezers once the solvent had reached the solvent line. The plate was then allowed to dry completely.
- Three types of solvent system were used based on difference in polarity for the detection of different compounds.
- The developed plate was then viewed under UV light for the detection of bands and spots.

### Charring of TLC plates

**Materials**

- Tweezers.
- 2% ninhydrin
- Ethanol solution.
- Hot plate.
- Petri dish.
- $H_2SO_4$
- Anisaldehyde

Spreading Procedure of TLC plate of Protein test

- 1 gm ninhydrin was added in 50 ml ethanol to produce 2% ninhyrdin solution.
- 1 ml of ninhydrin solution was added to 9 ml of ethanol which was taken in a Petri dish.

- The TLC plate was dipped in this solution using tweezers with the silica face down.
- The plate was left in the open for 10 minutes to allow for drying.
- A hot plate was heated to about 90 degree C and the plates were heated until the spots developed.

Spreading Procedure of TLC plate of Carbohydrate test

- 1 drop anisaldehyde added with 5 ml Distil $H_2O$.
- 10µl $H_2SO_4$ added in this solution which was taken in a Petri dish.
- The TLC plate was dipped in this solution using tweezers with the silica face down.
- The plate was left in the open for 10 minutes to allow for drying.
- A hot plate was heated to about 90 degree C and the plates were heated until the spots developed.( Saha, et.al.,2014)

**C. Anti-oxidant tests:**

- DPPH Charring Process Of TLC Plate

**Materials Required**

- 4% DPPH stock solution (1%), Methanol (9 ml).
- Test Tube.
- Pipette.
- Pipette filter.
- Petri dish and Tweezers.

**Procedure**

- 0.4% solution of DPPH was prepared by adding 9 ml of methanol to 1 ml of 4% DPPH stock solution. The procedure was carried out in a dark room as DPPH is light sensitive.
- By using tweezers the developed TLC plates would be dipped into this solution on the silica face down.
- The plates were left in the dark room for 30 minutes for the color to develop after which they were observed for the formation of yellow, golden / brown color on the background

of purple. This coloration indicates the presence of compounds that have antioxidant properties (Milena, N., 2011).

**Principle of DPPH test:**

This is known as a standard 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay. The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. Figure below, shows the mechanism by which DPPH• accepts hydrogen from an antioxidant. DPPH• is one of the few stable and commercially available organic nitrogen radicals (1). The antioxidant effect is proportional to the disappearance of DPPH• in test samples. Monitoring DPPH• with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH• shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm. (J. Lewis, 2012)

**Equipment and Materials Required**

1. Test tube
2. Foil paper
3. Micro pipette
4. UV Spectometer
5. Mortar & pestle
6. Filter paper
7. Funnel
8. Beaker
9. DPPH
10. Methanol
11. Distill water

**Procedure**

a) The DPPH radical scavenging method was used for the determination of the antioxidant capacity of the extracts. Different concentrations of the plant extract were added to methanol solution of DPPH.

b) Different concentrations of L-Ascorbic acid (2-10 mg/ml) were used as the standard antioxidant.

c) After 30 min at room temperature, the absorbance values were measured at 517 nm on a spectrophotometer and converted into the percentage antioxidant activity using the equation,

DPPH antiradical scavenging capacity (%) = [(Ab. of control – Ab. of sample)/Ab. of control] × 100.

d) Different concentrations of plant extract solution was used as a blank, while DPPH solution plus methanol was used as a control.

e) The IC50 values were calculated by the sigmoid non-linear regression model using plots, where the abscissa represented the concentration of tested plant extracts and the ordinate the average percent of scavenging capacity. IC50values denote the concentration of the sample required to scavenge 50% of DPPH radicals.

Standard Preparation:

1) 250 mg Ceevit Tablet (Square) was crushed in mortar and pestle.

2) 100 ml distilled water was added and the solution was filtered.

3) It was then diluted by 10 times (2 ml of the filtered solution was taken and 18 ml water added).

4) The solution was taken in 5 test tubes to prepare 5 different concentrations.

5) 1ml, 2ml, 2ml, 4ml and 5ml solution were taken in 5 different test tubes and the volume adjusted to 5 ml with water in all the test tubes.

Sample Preparation:

1) 100 µL of extracted sample was added in to first test tube which contain 2 ml methanol.

2) From the first test tube 1ml solution was taken in to second test tube which contain 1 ml methanol.

3) The procedure was continued for other 3 test tube for having total five different concentrations.

4) 1ml solution was discarded from the last test tube.

5) The volume was adjusted to 4 ml with methanol in all the test tubes.

Blank Preparation:

Blank was prepared by adding 4 ml methanol in a test tube. In all the test tubes 100 µL DPPH solution was added in dark and left for 30 minutes. After that UV absorbance was measured in UV machine at 517 nm.

D. **Antimicrobial Screening of VLC Fraction by using Agar Diffusion Method**

**Principle of Disc diffusion method:**

When a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition".

**Equipment and Materials Required**

1. Petri dish 2. Disc

3. Watch glass 4. Tweezers

5. Micro pipette
6. Autoclave machine
7. Laminar air flow
8. Incubator
9. Spreader
10. Spatula
11. Ethanol
12.Nutrient agar
13. Distill water
14.Sodium chloride
15. Antibiotic (Ciprofloxacin)

Test Organisms

I. **Gram Negative Bacteria**

- *Salmonella typhi*
- *E.coli*
- *Pseudomonas*
- *Shigella dysentery*
- *Salmonella paratyphi*
- *Vibriomimicus*
- *Shigella boydii*
- *Kleb siella*

II. **Gram Positive Bacteria**

- *Staphylococcus aureus*
- *Streptococcus pyrogeny*
- *Beta Hemolyte streptococcus*
- *Bacillus cereus*
- *Bacillus subtilis*

III. **Fungi**

- *Candia albicans*
- *Bacillus megaterium*
- *Saccharomyces cerevisiae*
- *Aspergillus niger*

- The Culture Medium and Its Composition

Nutrient agar was used to conduct the antimicrobial screening using the disc diffusion method. The nutrient agar was bought from the market. Nutrient agar contains the following substances:

❖ Ingredients

- Bacto peptone - 0.5gm
- Sodium chloride - 0.5gm
- Bacto yeast extract - 1.0gm
- Bacto agar - 2.0gm
- Distilled water (Qs) - 100ml

❖ Preparation of the Medium

First of all, the amount of nutrient agar needed was calculated and then added to distilled water in an agar bottle and mixed thoroughly. It was then autoclaved to dissolve the agar and sterilize it.

❖ Sterilization Procedure

Sterilization is a term referring to any process that eliminates (removes) or kills all forms of life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present in a specified region, such as a surface, a volume of fluid, medication, or in a compound such as biological culture media. In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121ºC and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

❖ Preparation of the Test Plates

a. The test organisms were transferred from the subculture to petri dish containing the required amount of melted and sterilized agar medium as required by the size of the dish.
b. The bacterial and fungal suspension was taken by a loop and mixed with normal saline with the help of vortex machine.
c. Then a sterilized cotton bud was taken and dipped into the bacterial/ fungal suspension. Then the bacterial/fungal sample is applied to the Petri dish with the help of this cotton bud.
d. The swabbing was done carefully so that the microorganisms would be spread out evenly on the dish.

❖ Standard Discs

These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation Ciprofloxacin (30µg/disc) standard disc was used as the reference.

❖ Blank Discs

These were used as negative controls, which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves. Here the negative control used was methanol.

❖ Preparation of sample discs with test samples

a. In a specific volume of solvent, measured amount of each test sample was dissolved to obtain the desired concentrations in an aseptic condition.
b. For the each extract of husk, a stock solution of 10mg/ml was prepared and was used directly.
c. Sterilized metrical filter paper discs were taken in a blank Petri dish under the laminar hood. Then discs were soaked with solutions of 10µl of test samples and dried.

**Procedure of Disc diffusion method:**

1. Six different bacterial strains of gram positive, six different strains of gram negative bacteria were used to carry out this assay.

2. Nutrient agar was used as the culture media. Stocks of these bacterial solutions were revived in nutrient agar by incubating at 370C for 24 hrs.

3. A single disk diffusion method was used to assess the presence of antimicrobial activities of the extract.

4. Whatman filter paper were punched, and 0.6 cm disks were collected in a beaker. The beaker with petri dishes, forceps, tips, spreader were covered with foil paper and autoclaved.

5.5 µl of extracted sample were loaded per disc with the help of micropipette.

Figure 4.7 Micropipette, pipette tips

6. The revived test organisms were spread onto nutrient agar plates by spreader.

7. The disc were then placed all plates. Standard disc of Ciprofloxacin (25µg/disc) was used as positive control.

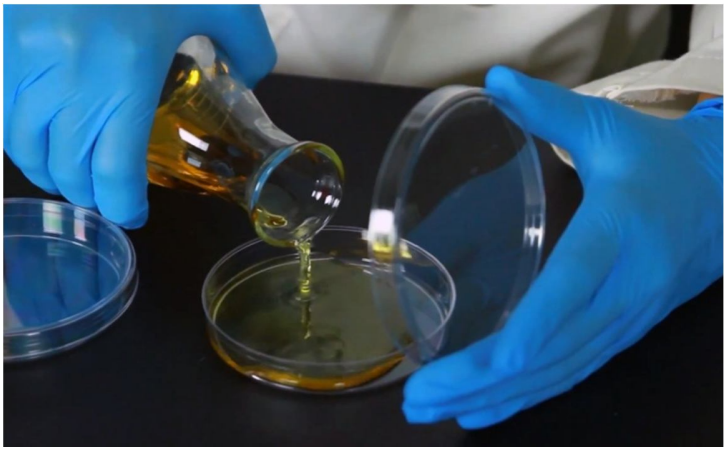


Figure 4.8 Agar media in plate.

8. After incubation at 370C for 24 hours, the antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm.

1 2

Figure 4.9 Incubator (1) of microbiology lab and laminar air flow (2).

**Minimum Inhibitory concentration of *Arachis hypogaea* cyclohexane primary VLC fraction**

The minimal inhibitory concentration (MIC) values, which represent the lowest extract concentration that completely inhibits the growth of microorganisms. The sample in question is first prepared by producing a standard stock solution then subsequently diluting it to obtain different concentration. Minimum Inhibitory concentration is carried out by using 100µL of suspension containing ~103 CFU/mL of microorganism spread on nutrient agar medium (Himedia,India).*Samonella paratyphii* and *Bacilus subtilis* were used to carry out this assay. Dried and sterilized filter paper discs (6 mm diameter), methanol extract of egg shell, a stock solution of 10 mg/ml was prepared. The plant samples were diluted in eppendorf tubes to get different concentrations were prepared and discs were soaked with each solutions of 10µl of test samples were placed gently on the previously marked zones in the agar plates. After incubation at $37^0$C for 24 hours, the antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The results were expressed as mean ± standard deviations.

**E. In vitro anti-diabetic test**

**Glucose uptake in Yeast cells**

The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts were added to 1mL of glucose solution and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µL of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and amount of glucose was estimated in the supernatant (Cirillo, 1962). The percentage increase in glucose uptake by yeast cells was calculated using the following equation:

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

Where, Abs=control is the absorbance of the control reaction (containing all reagents except the test sample)

Abs sample = the absorbance of the test sample. All the experiments were carried out in triplicates. (Mishra, Kavrekar and Nair, 2013)

**Equipment and Materials Required**

1. Test tube
2. Beaker
3. Funnel
4. Micropipette
5. Filter paper
6. Incubator
7. Centrifuge machine
8. UV spectrometer
9. Distill water
10. Glucose
11. Yeast
12. Metformin

Preparation of glucose solution:

2gm glucose was added to 5 ml distilled water. Required amount of glucose solution was prepared by this procedure.

Standard preparation:

1. 500 mg metformin Tablet was crushed in mortar and pestle.
2. 100 ml distilled water was added and the solution was filtered.
3. 500 µl filtered solution was taken in the first test tube which contain 1.5 ml glucose solution.
4. Then serial dilution was done to have 5 different concentrations.

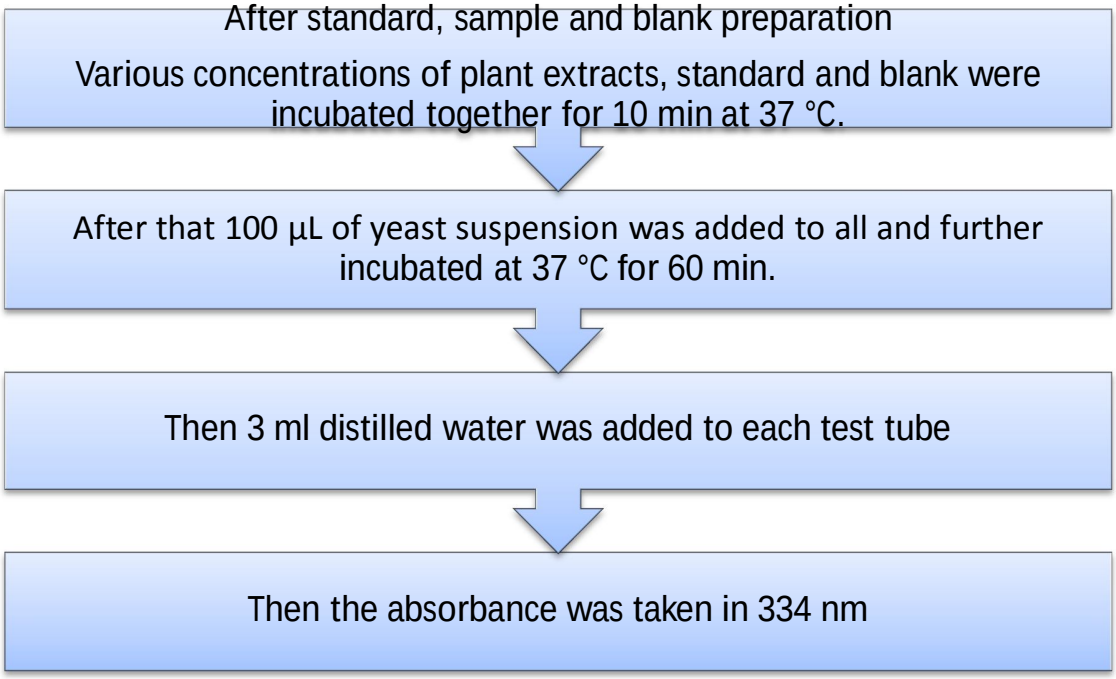
Sample preparation:

a. 100 µL of extracted sample was added in to first test tube which contains 2 ml glucose solution.
b. Serial dilution was done to have 5 different concentrations.

Blank preparation:

1ml glucose solution without sample and standard was used as blank.

Procedure:




**F. In vitro anti-inflammatory test**

Preparation of Human Red Blood Cells (HRBC) Suspension

Fresh whole human blood was collected and mixed with equal volume of sterilized phosphate buffer saline. The blood was centrifuged at 1000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.

Heat Induced Hemolysis

The principle involved here is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 100 $\mu$l HRBC suspension [10 % v/v] with 100 $\mu$l of plant extracts and standard drug diclofenac sodium of various concentrations and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37 oC for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm. (Chippada et al., 2011)

$$\text{Percentage Stabilization} = \frac{\text{Absorbance of control} - \text{Absorbance of Test sample}}{\text{Absorbance of control}} \times 100$$

(Kar et al., 2012)

**Equipment and Materials Required**

1. Test tube
2 Beakers
3. Funnel
4. Filter paper
5. Micro pipette
6. Measuring cylinder
7. Heparin tube
8. UV spectrometer
9. Incubator
10. Centrifuge machine
11. Na2HPO4
12. NaH2PO4
13. NaCl
14. Diclofenac sodium
15. Distill water

Standard preparation:

a. 50 mg diclofenac sodium tablet was crushed in mortar and pestle.
b. 10 ml distilled water was added and the solution was filtered.
c. 100 µl filtered solution was taken in the first test tube which contain 2 ml phosphate buffer saline.
d. Then serial dilution was done to have 5 different concentrations.

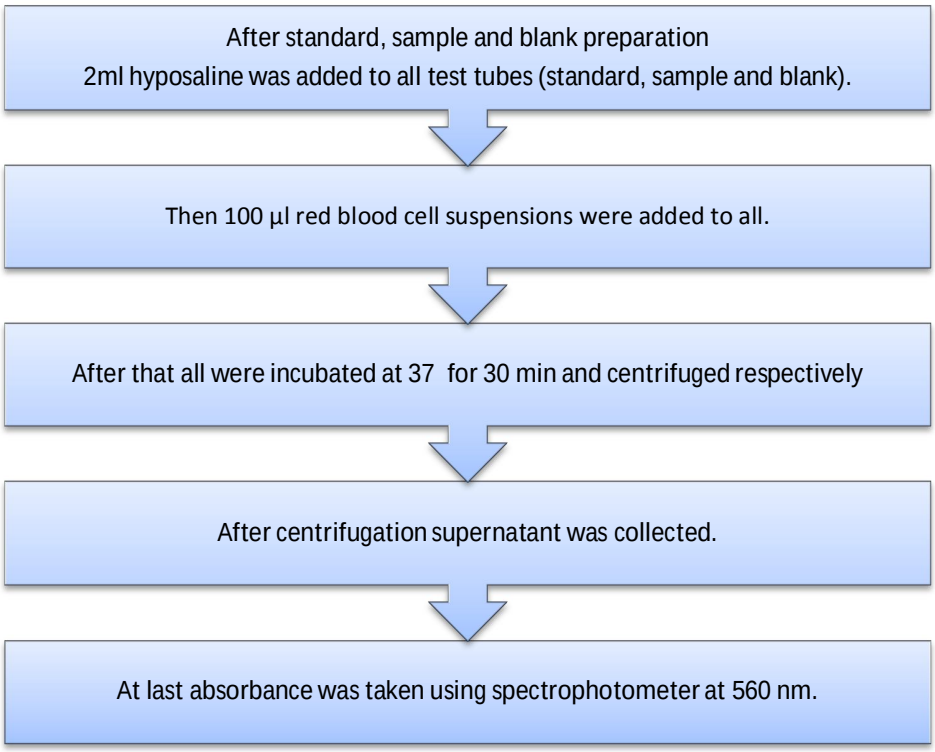
Sample preparation:

a. 100 µl of extracted sample was added in to first test tube which contains 2 ml phosphate buffer saline
b. Serial dilution was done to have 5 different concentrations.

Blank preparation:

1ml phosphate buffer saline without sample and standard was used as blank.

Procedure:




## G. Hemagglutination (HA) Assay

**Principle:**

The hemagglutination assay is a method for 71ittering influenza viruses based on their ability to attach to molecules present on the surface of red blood cells. A viral suspension may agglutinate the red blood cells, thus preventing them from settling out of suspension. By serially diluting a virus in a 96-well plate and adding a consistent amount of red blood cells, an estimation of the amount of virus present can be made.

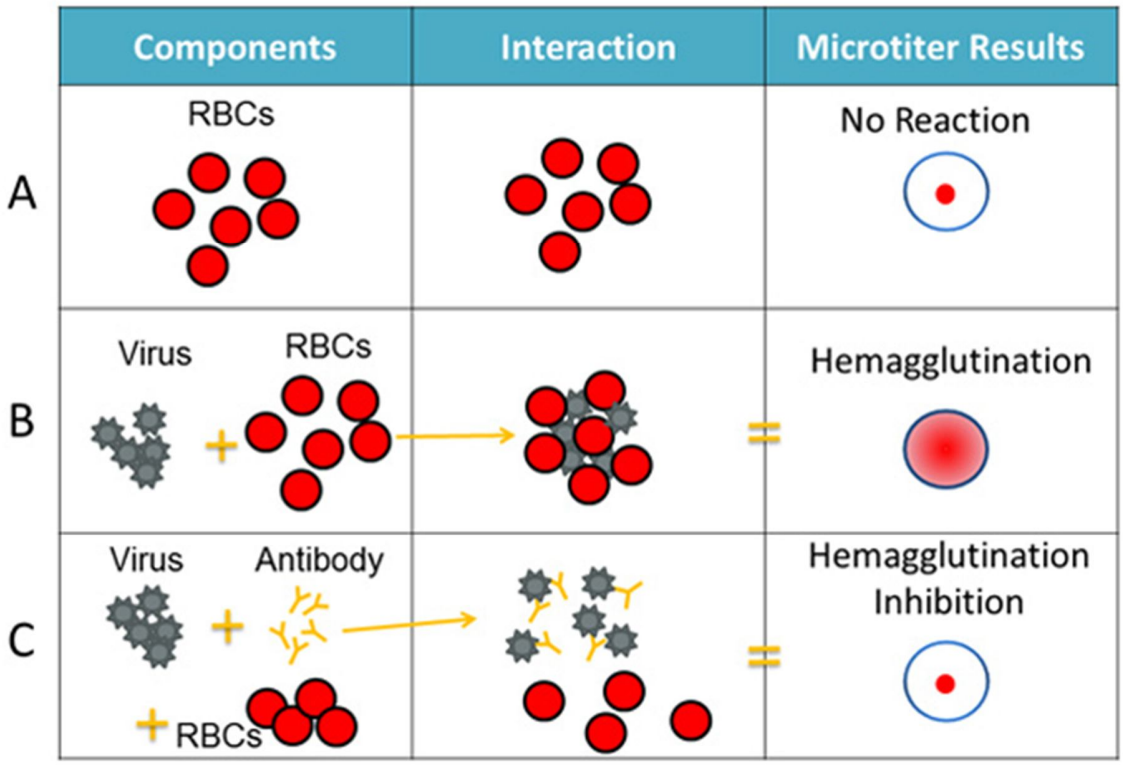



Figure 4.10 Mechanism of heamaglutination

**Equipment and Materials Required**

1. U- or V-bottom microtiter plates
2. Micropipette with tips
3. Phosphate buffered saline (0.01M, pH 7.2)
4. Washed human erythrocytes (0.5%)

5. Centrifuge machine

(Hemagglutination Assay (HA) and Hemagglutination Inhibition Assay (HI): AI and NDV, 2006)

RBC preparation:

1. 4 ml of human blood was pipetted into a 14 ml test tube and topped off with PBS.

2. Centrifuged at 1000 rpm for 10 minutes.

3. The supernatant was aspirated without disturbing the blood cells.

4. 12 ml PBS was added and mixed by inverting.

5. It was centrifuged again at 1000 rpm for 5 minutes and washed two more times.

6. Supernatant was aspirated after final wash and enough PBS was added to make a 10% solution of red blood cells.

7. Final working solution of 0.5% RBCs was made in PBS.

**Procedure:**

1. A round-bottomed 96-well dish is preferred for this assay. Flat-bottomed plates will also work, but need to be placed at an incline to develop.

2. To each 50 µL PBS was added.

3. In the first column, 50 µl of sample was added.

4. After mixing each well and transferred 50 µl to the next well on its right. Repeated mixing and transferring of 50 µl down the length of the plate and the last well 50 µl was discarded.

5. 50 µl of 0.5% red blood cell was added to each well and mixed gently.

6. The plate was kept at room temperature for 30-60 minutes to develop.

(Pankaj, 2015)

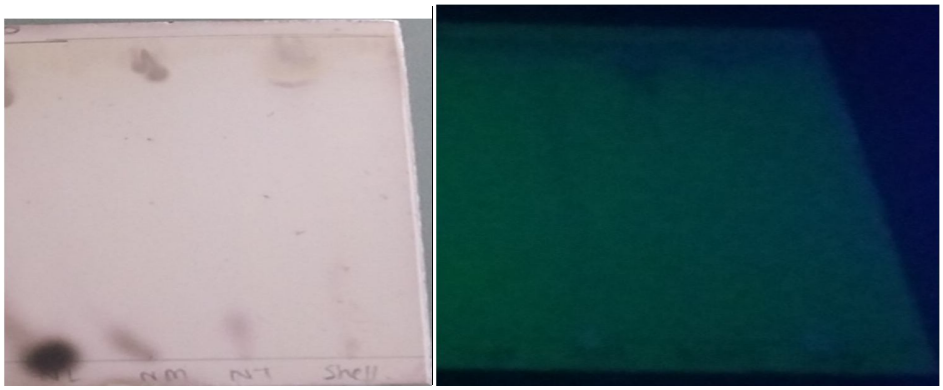
# UNIT FIVE: RESULTS

## RESULTS

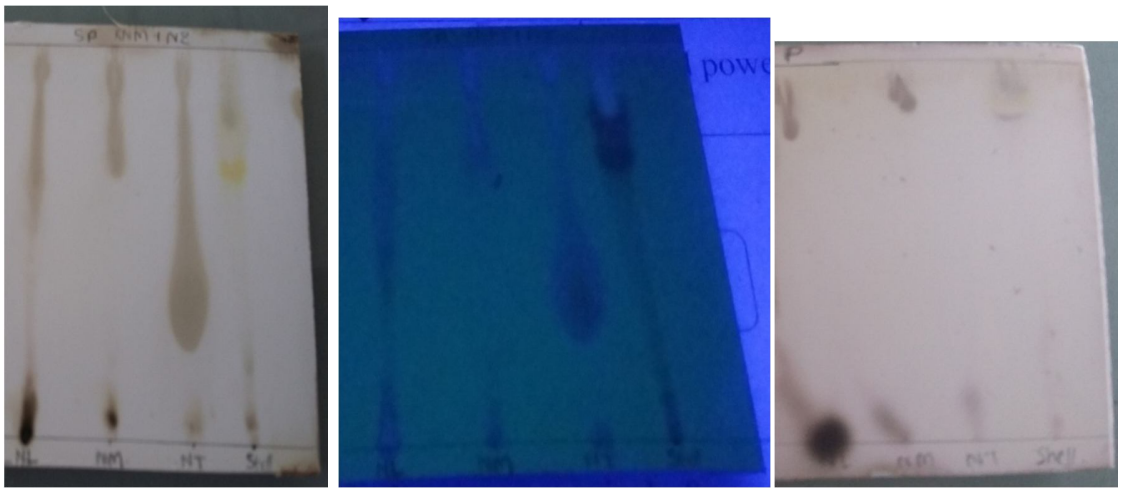
### Thin layer chromatography

TLC plates were developed with petroleum ether, cyclohexane, xylene, dichloromethane, chloroform and methanol fraction of all variety of *Arachis hypogaea* using solvent system-1 polar basic solvent (ethyl acetate, ethanol, water in the ratio of 8:1.2:0.8) solvent system- 2 semi polar solvent (benzene, ethyl acetate in the ratio of 6:4) and solvent system-3 non polar basic solvent (Benzene, ethanol, ammonium hydroxide in the ratio of 9:1:0.1). The best result was found using solvent system-2 semi polar solvent (benzene, ethyl acetate in the ratio of 6:4).The same procedure was followed with lectin sample of both shell and nut layer.

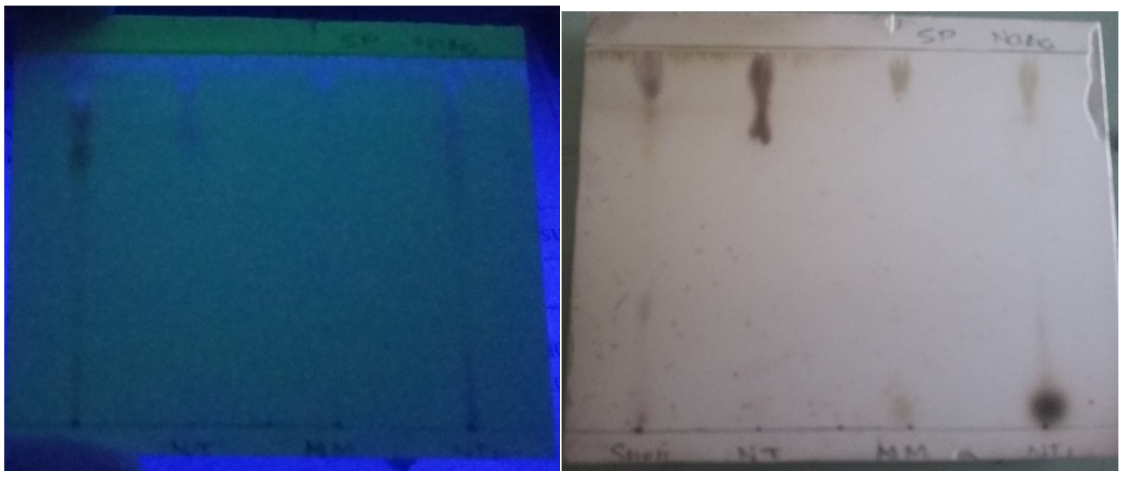
**Thin layer Chromatography of Shell and Nut layers (upper, middle and lower) of *Arachis hypogaea* extract in polar solvent.**



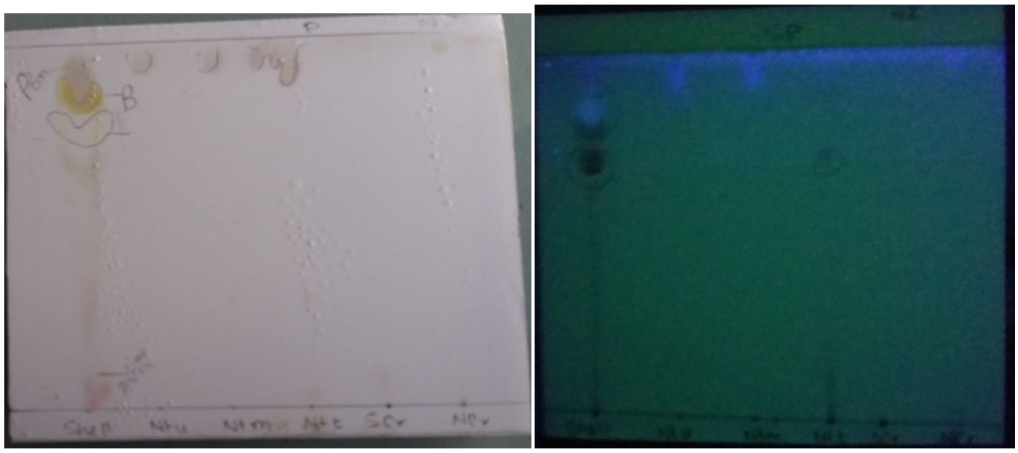
**Thin layer Chromatography of Shell and Nut layers (upper, middle and lower) of *Arachis hypogaea* extract in semi polar solvent.**



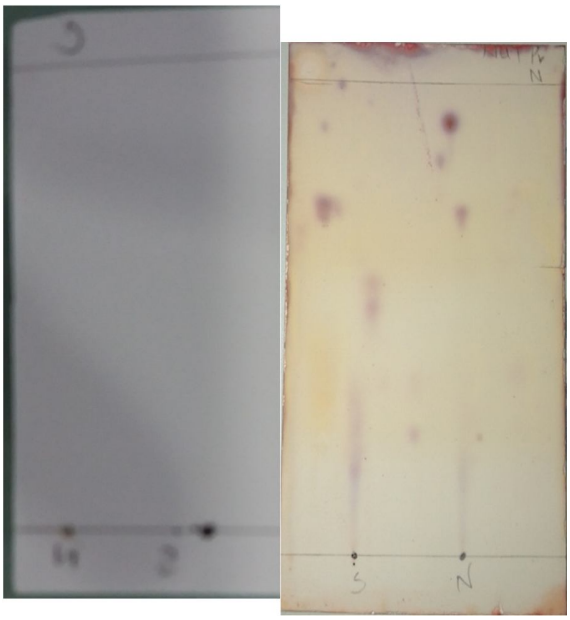
**Thin layer Chromatography of Shell and Nut lower layer fractions (primary VLC fractions) of *Arachis hypogaea* extract**



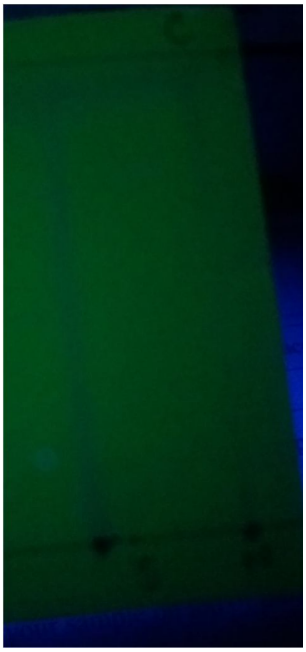
**Thin layer Chromatography of Shell and Nut lower layer fractions (primary VLC fractions) of *Arachis hypogaea* extract in polar solvent**



**Thin layer Chromatography of Shell and Nut Lectin fractions of *Arachis hypogaea* extract**



**Thin layer Chromatography of Shell and Nut Lectin fractions of *Arachis hypogaea* extract under ultraviolet light**



**DPPH radical scavenging activity:**

The result of antioxidant test of different fraction of *Arachis hypogaea* are given below-

Table 3.1 Antioxidant activity of standard Ascorbic acid.

| Sample Name | Concentrations (mg) | Absorbance (517 nm) | % of inhibition |
|---|---|---|---|
| Ascorbic acid | 1 | 0.027 | 77.2287 ± 1.45 |
| | 0.8 | 0.033 | 72.09615 ± 1.26 |
| | 0.6 | 0.0375 | 68.29622 ± 1.04 |
| | 0.4 | 0.0415 | 64.93107 ± 1.20 |
| | 0.2 | 0.0455 | 61.5942 ± 1.17 |

% Inhibition

Figure 4.9 % Free radical scavenging activity of ascorbic acid

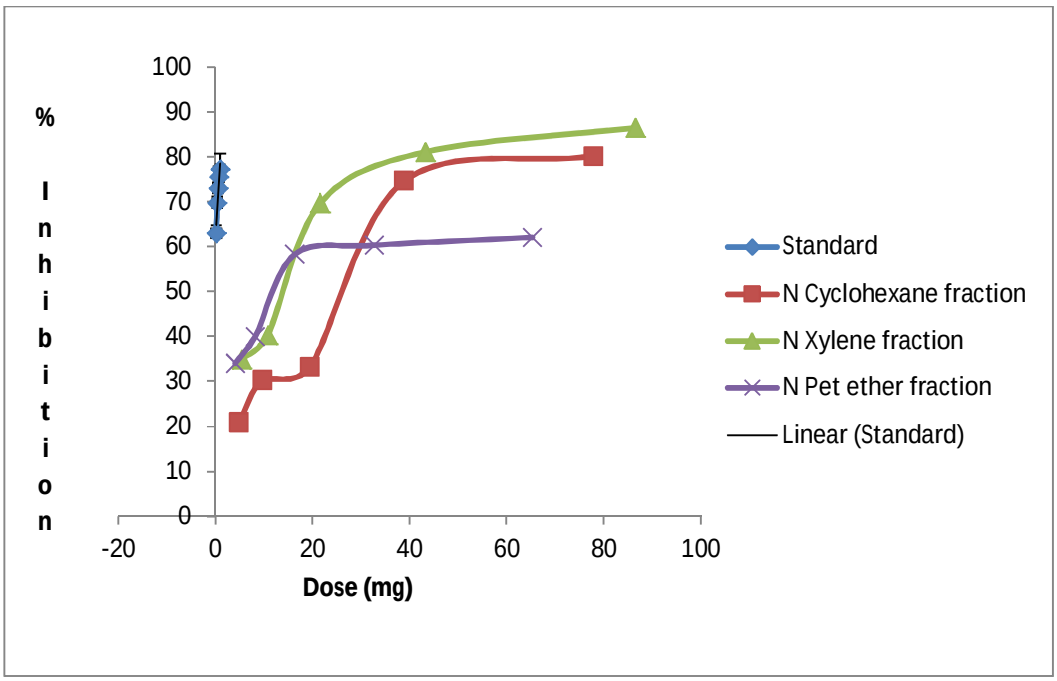



Figure 4. 10 % Free radical scavenging activity of nut lower layer cyclohexane, xylene, pet ether fractions of *Arachis hypogaea* and ascorbic acid.

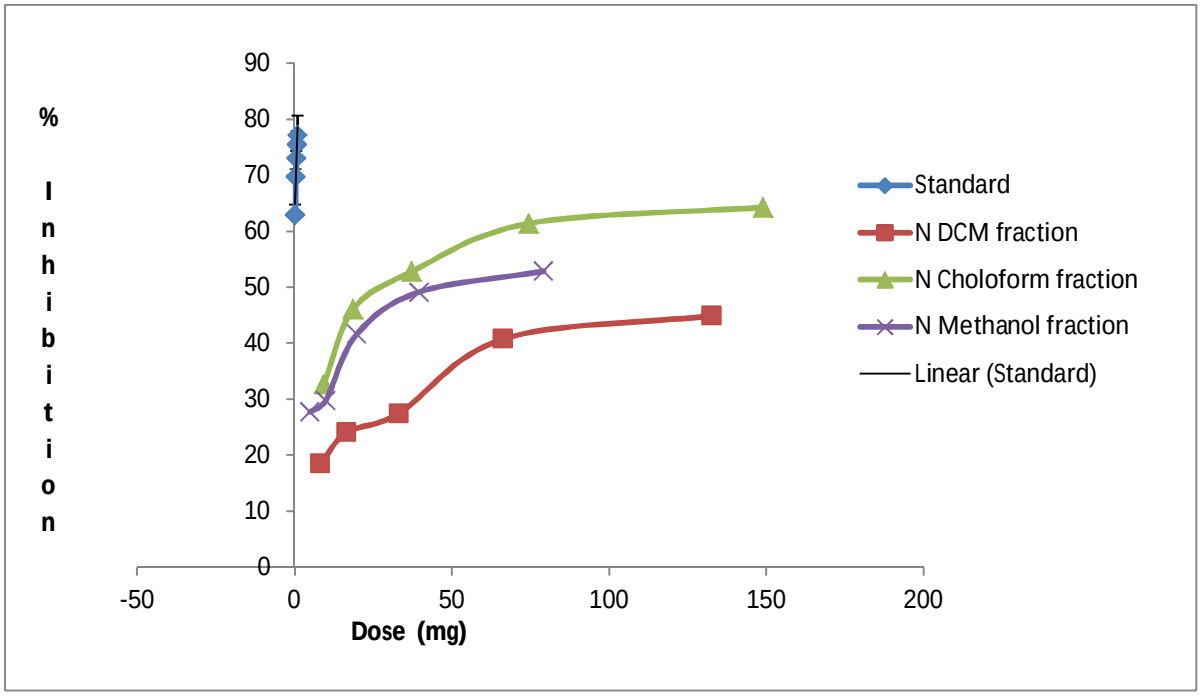



Figure 4.11 % Free radical scavenging activity of nut lower layer chloroform, dichloromethane, methanol fractions of *Arachis hypogaea* and ascorbic acid.

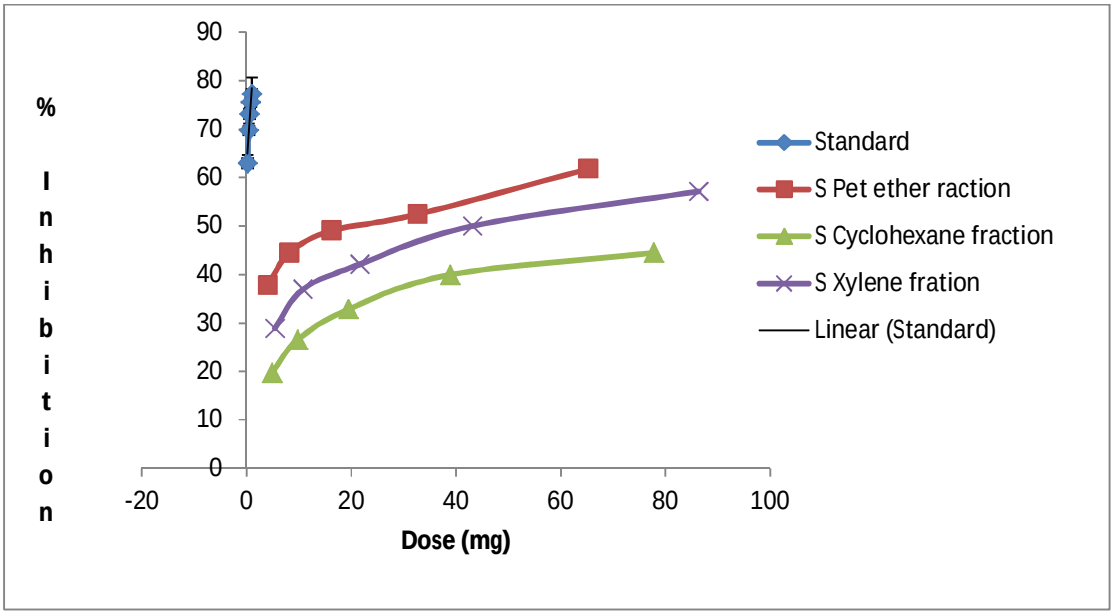



Figure 4.12 % Free radical scavenging activity of shell pet ether, cyclohexane and xylene fractions of *Arachis hypogaea* and ascorbic acid.

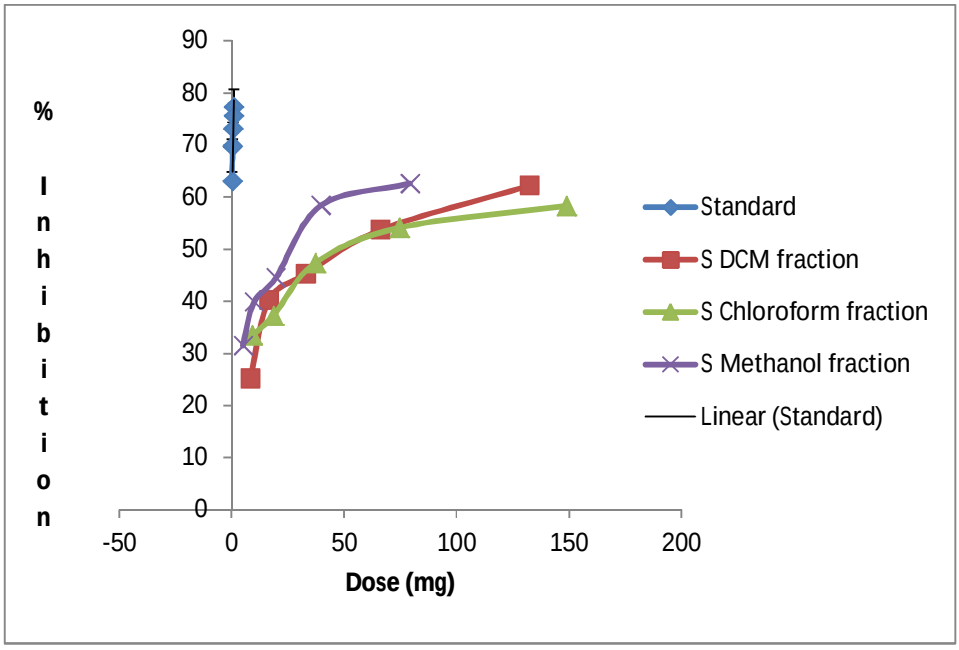



Figure 4.13 % Free radical scavenging activity of shell dichloromethane, chloroformand methanol fractions of *Arachis hypogaea* and ascorbic acid.

**Anti – diabetic activity:**

The result of hypoglycemic test of different fraction of *Arachis hypogaea* given below-

Table 3.2 Antidiabetic activity of standard metfrormin.

| **Sample Name** | **Concentrations (mg)** | **Absorbance (334 nm)** | **% of inhibition** |
|---|---|---|---|
| Metformin | 2.5 | 0.732 | 11.67013 ± 1.60 |
| | 1.2 | 0.7075 | 8.628617 ± 0.96 |
| | 0.624 | 0.6895 | 6.413319 ± 1.01 |
| | 0.312 | 0.671 | 3.989987 ± 1.56 |
| | 0.156 | 0.639 | 0 ± 1.24 |

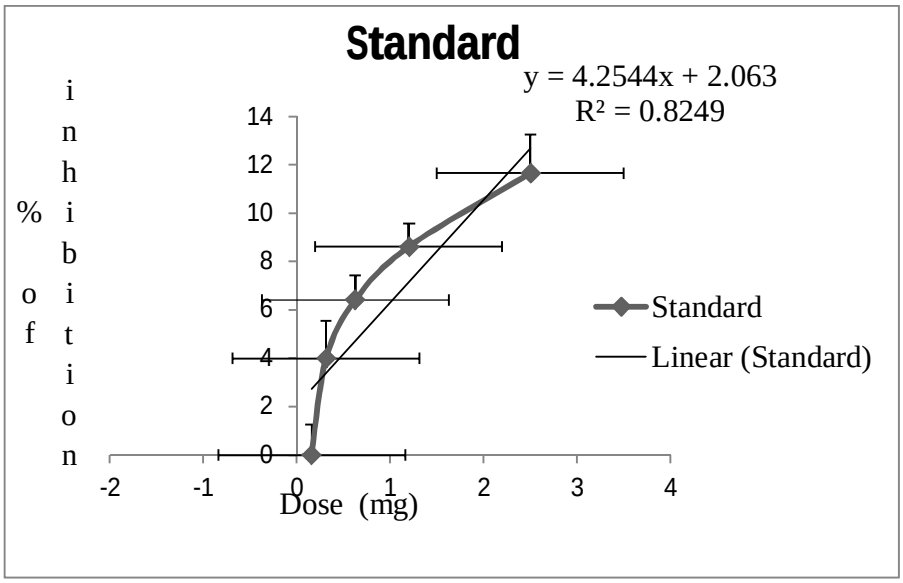



Figure 4.14 Anti-diabetic activity of Metformin

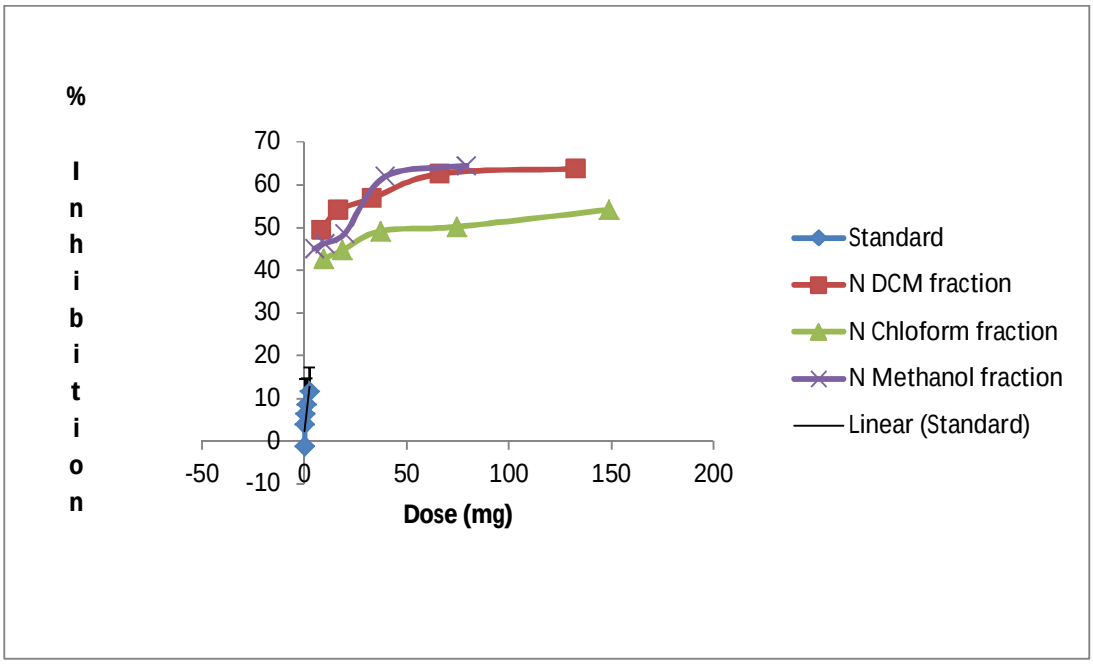



Figure 4.15 Anti – diabetic activity of nut lower layer chcloroform, dichclоromethane, methanol fractions of *Arachis hypogaea* and metformin

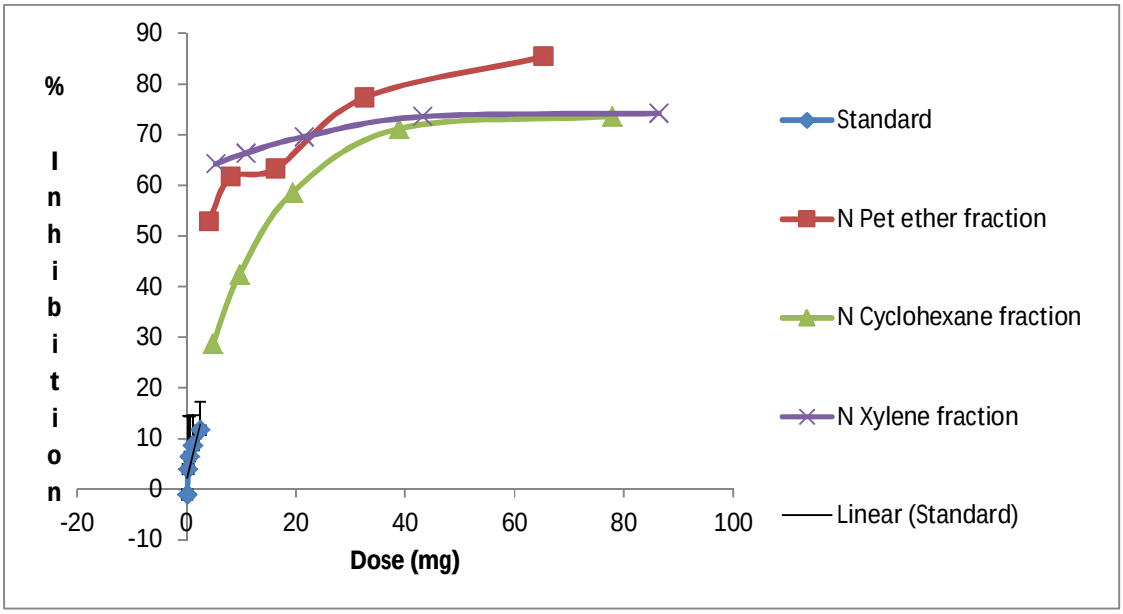



Figure 4.16 Anti – diabetic activity of nut lower layer pet ether, cyclohexane, xylene fractions of *Arachis hypogaea* and metformin

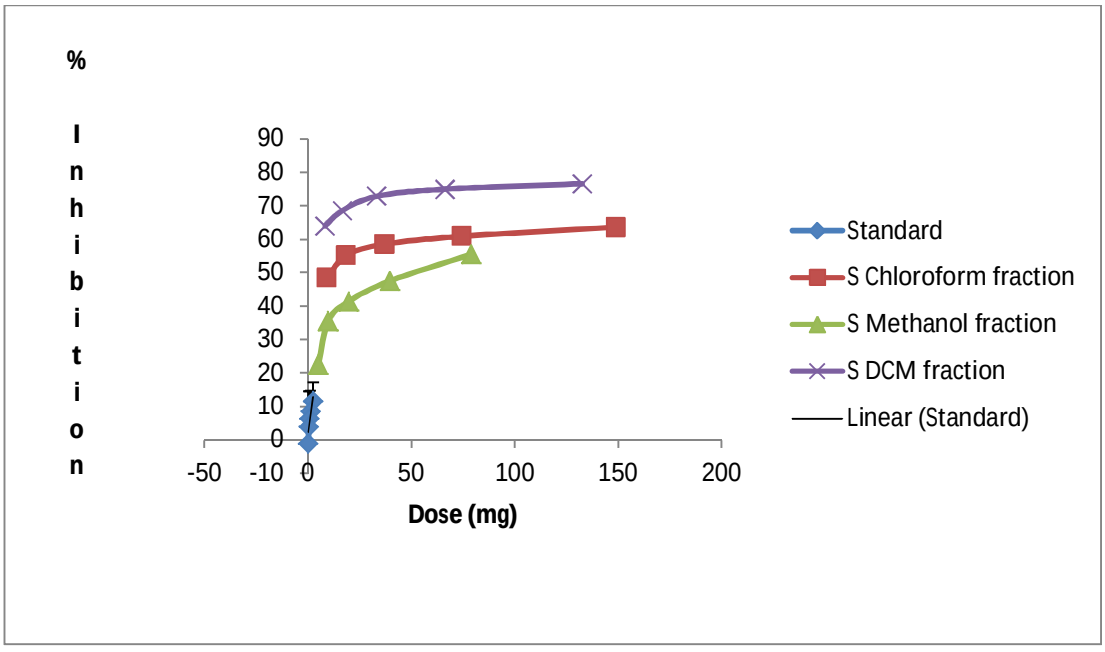


Figure 4.17 Anti – diabetic activity of shell chloroform, methanol, dichloromethane fractions of *Arachis hypogaea* and metformin

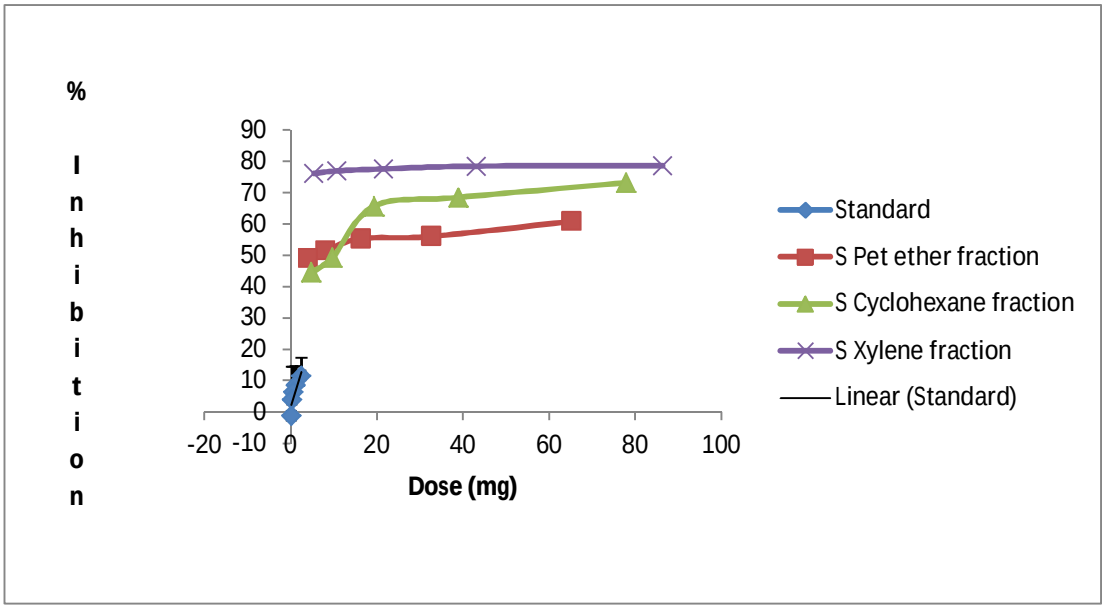



Figure 4.18 Anti – diabetic activity of shell pet ether, cyclohexane, xylene fractions of *Arachis hypogaea* and metformin

**Anti-bacterial activity:**

20µl of samples were used against 500µl of spreading pathogenic bacteria in agar plate and kept overnight. Zone of inhibition was observed and measured by Varner scale after 24hrs of incubation. The zones of inhibition were measured against strains of Gram positive, Gram negative bacteria and fungi. The positive control used was ciprofloxacin (30µg/disc).Petroleum ether, cyclohexane, and xylene, dichloromethane, chloroform and methanol fractions of all variety of *Arachis hypogaea* was used to evaluate the activity against different types of microorganism. Bacteria were considered for antimicrobial activities those passed the gram staining and

catalyses test.

Table 3.3 Antibacterial activity of the methanolic extract of *Arachis hypogaea* shell VLC fraction of petether, cyclohexane, Xylene, DCM,Chloroform and methanol against gram positive bacteria.

| BACTERIA | | ZONE OF INHIBITION | | | | |
|---|---|---|---|---|---|---|
| | Cyclohexane | Control | Xylene | Control | DCM | Control |
| Bacillus megaterium | 1.2±0.282 | 3 ±0.070 | - | - | - | - |
| *Bacillus subtilis* | 1.2±0.141 | - | - | - | - | - |
| *Bacillus cereus* | 0.85±0.070 | 2.95±0.070 | | 0.85±0.212 | 0.85±0.212 | 2.9 |
| *Candida albicans* | - | - | - | - | - | - |
| *Staphylococcus aureus* | 0.75±0.070 | 2.3±1.41 | - | - | - | - |
| *Sarcina lutea* | - | - | - | - | - | - |

Table 3.4 Antibacterial activity of the methanolic extract of *Arachis hypogaea* shell VLC fraction of petether, cyclohexane, Xylene, DCM,Chloroform and methanol against gram negative bacteria.

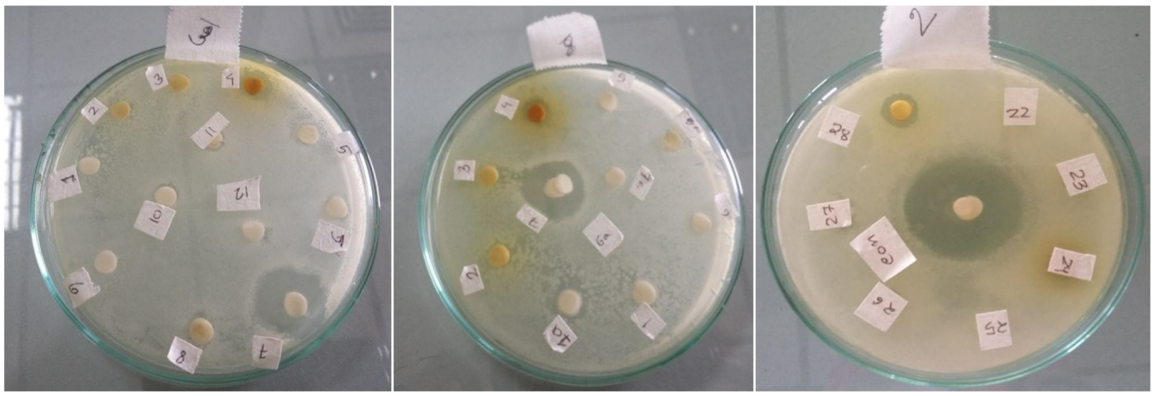
| BACTERIA | ZONE OF INHIBITION | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cyclohexane | Control | Xylene | Control | DCM | Control | Methanol | Control | Petether | Control |
| *Escherichia coli* | - | - | - | - | 0.75±0.070 | 2.6 | - | - | - | - |

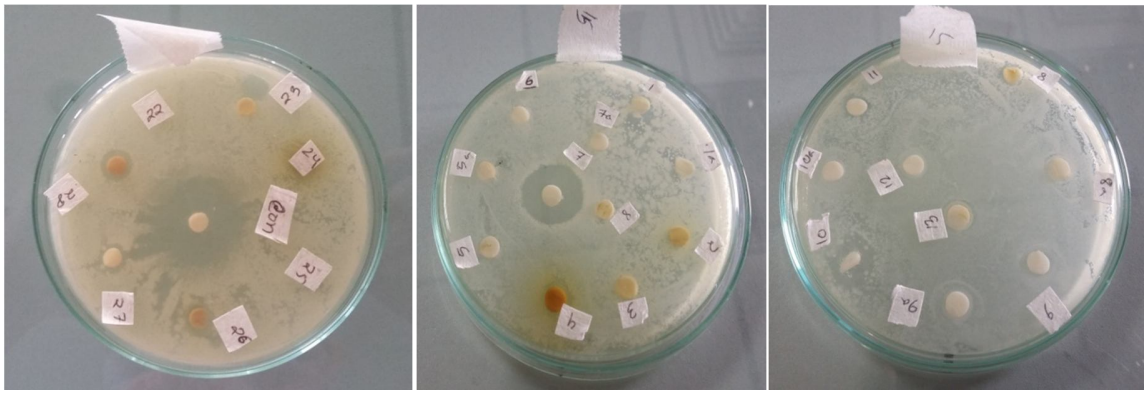
| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Vibrio parahemolyticus* | 1.35±0.353 | 2. ± 0.424 | - | - | - | | - | - | - | - |
| *Vibrio mimicus* | 1.45±0.212 | 2.45±0.070 | 0.85±0.070 | 3.2±0.283 | - | - | 0.85±0.070 | 2.1±0.070 | 1.05±0.070 | 3.2 |
| *Salmonella paratyphi* | 1.3±0.141 | 2.2 | - | - | - | - | - | - | - | - |
| *Salmonella typhi* | 1.05±0.353 | 3.25±0.070 | - | - | - | - | - | - | - | - |
| *Pseudomona aureaus* | 1.55±0.070 | 2±0.070 | - 0.65±0.070 | 1.45±0.778 | 0.8±0.141 | 2.2±0.282 | - | - | - | - |
| *Shigella dysenteriae* | 1.35±0.070 | 2.25±0.070 | - | - | - | - | | | | |

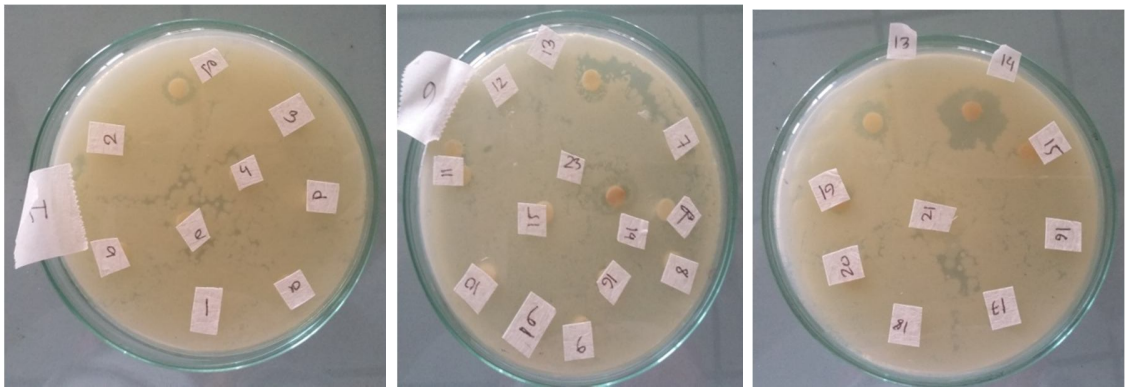
Table 3.5 Antibacterial activity of the methanolic extract of *Arachis hypogaea* nut lower layer VLC fraction of petether, cyclohexane, Xylene, DCM,Chloroform and methanol against gram positive and negative bacteria.

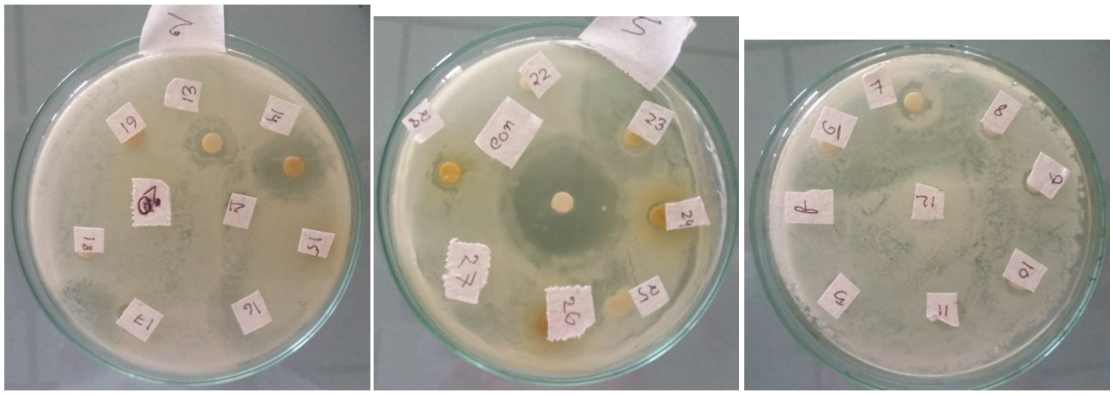
| BACTERIA | | ZONE OF INHIBITION | | | | |
|---|---|---|---|---|---|---|
| | Cyclohexane | Control | Xylene | Control | DCM | Control |
| *Bacillus megaterium* | - | - | - | - | - | - |
| *Bacillus subtilis* | - | - | - | - | - | - |
| *Bacillus cereus* | 0.85±0.070 | 2.9 | | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Candida albicans* | - | - | - | - | - | - |
| *Staphylococcus aureus* | 1.55±0.070 | 2.4 | - | - | 1.55±0.070 | 2.4 |
| *Sarcina lutea* | - | - | - | - | - | - |
| *Escherichia coli* | 0.75±0.070 | 2.7±0.141 | - | - | - | - |
| *Vibrio parahemolyticus* | - | - | - | - | - | - |
| *Vibrio mimicus* | - | - | - | - | 0.91±0.014 | 3.3±0.14 |
| *Salmonella paratyphi* | 1.25±0.212 | 2.25±0.070 | - | - | - | - |
| *Salmonella typhi* | - | - | 0.91±0.041 | 3.3±0.14 | - | - |
| *Pseudomona aureaus* | 1±0.141 | 2 | - | - | - | - |
| *Shigella dysenteriae* | - | - | - | - | - | - |









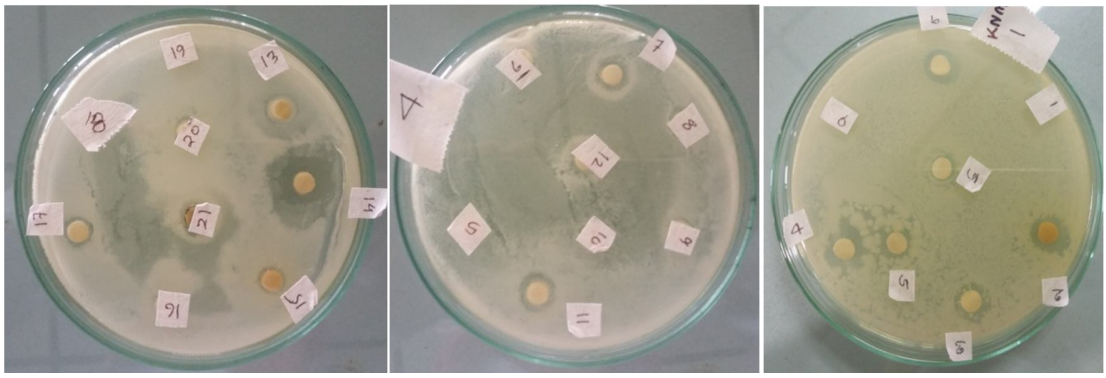


Figure 4.19 Different zones of inhibition of different VLC fractions

1= Shell Cyclohexane

2= Shell Xylene

3= Shell DCM

11= Nut Lower Layer Cyclohexane

4= Shell Methanol

13= Nut Lower Layer Xyene

5= Shell pet ether

14= Nut Lower Layer DCM

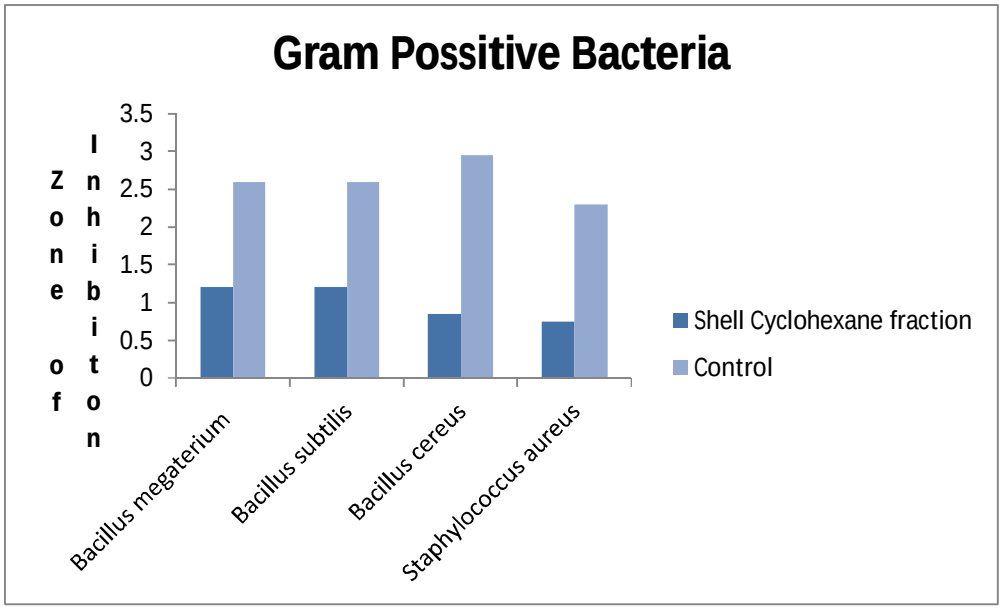



Figure 4.20a Zone of Inhibition of shell cyclohexane fraction *Arachis hypogaea*against gram positive bacteria

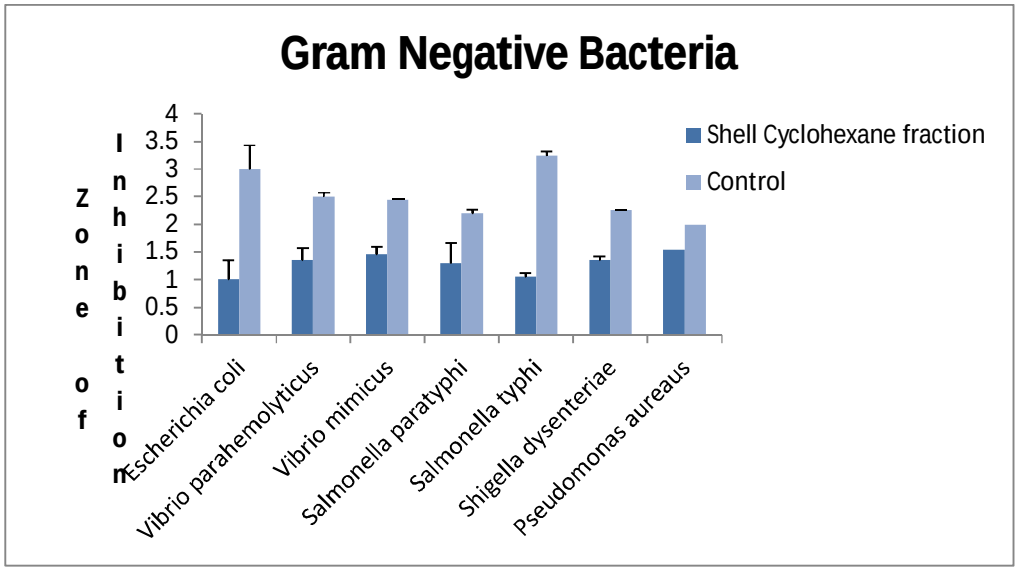



Figure 4.20b Zone of Inhibition of shell cyclohexane fraction *Arachis hypogaea* against gram negative bacteria

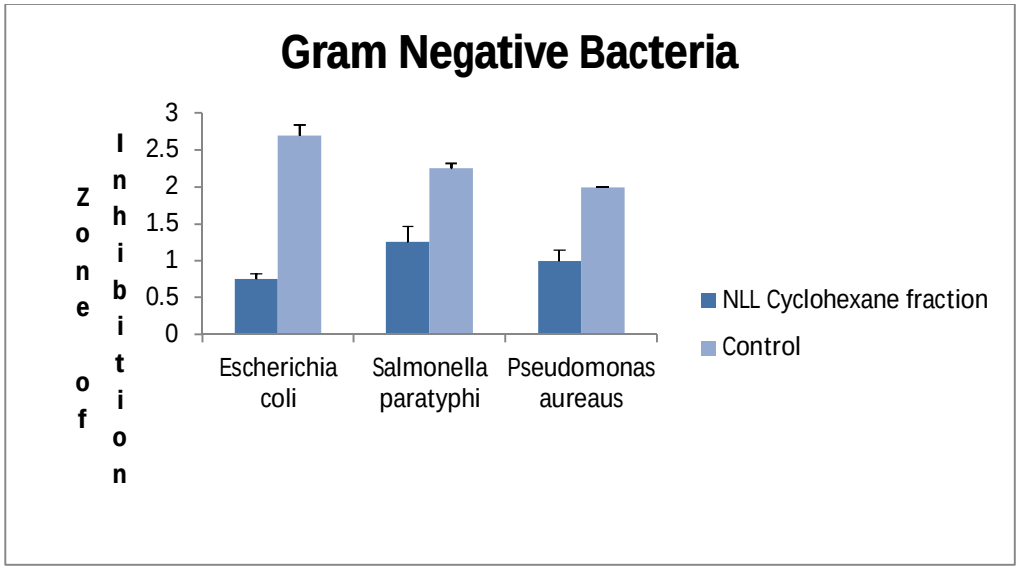



Figure 4.20c Zone of Inhibition of nut lower layer cyclohexane fraction *Arachis hypogaea* against gram negative bacteria

**Minimum Inhibitory Concentration:**

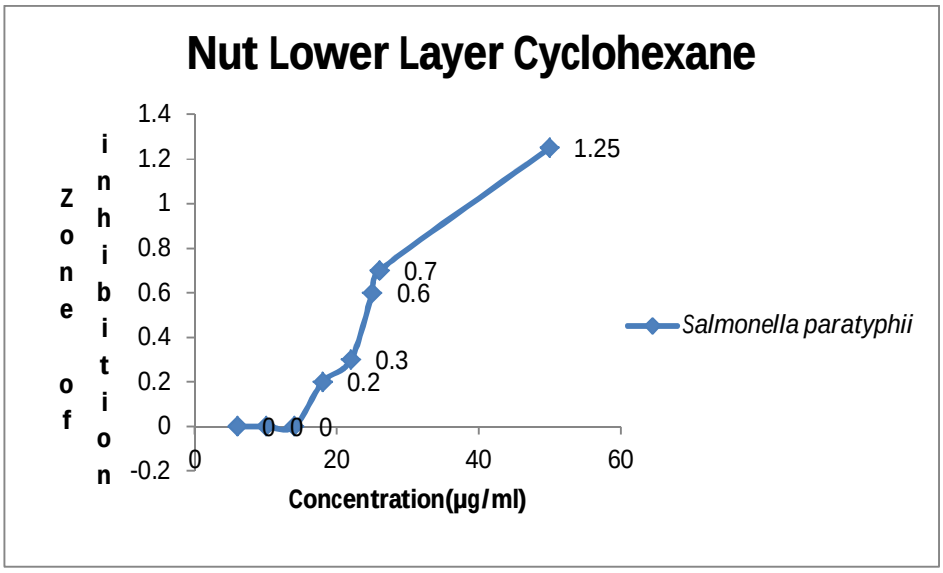



Figure 4.21d Minimum Inhibitory concentration of nut lower layer cyclohexane primary VLC fraction of *Arachis hypogaea* against *Salmonella paratyphii*

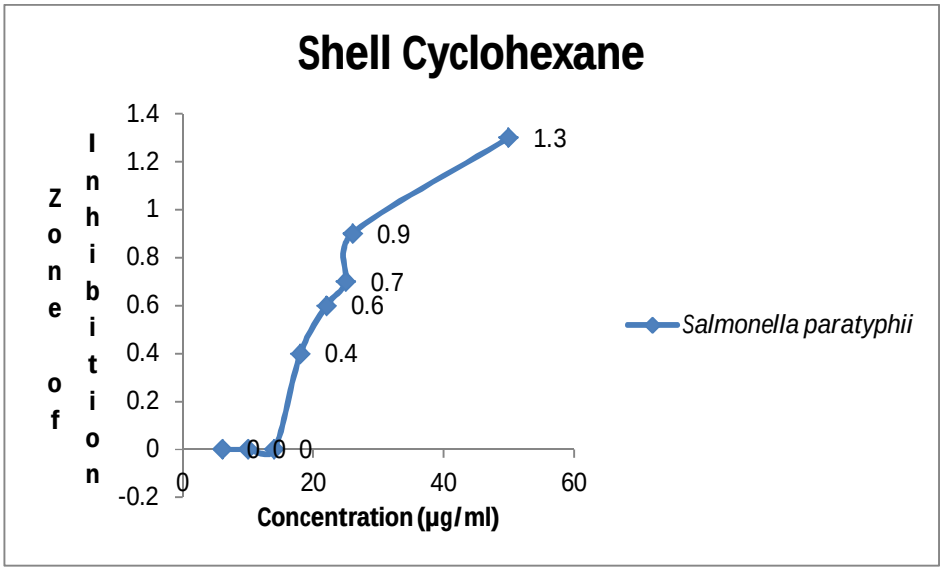



Figure 4.21e Minimum Inhibitory concentration of shell cyclohexane primary VLC fraction of *Arachis hypogaea* against *Salmonella paratyphii*

**Anti-inflammatory activity:**

The result of anti-inflammatory test of different fraction of *Arachis hypogaea* are given below-

Table 3.6 Anti- inflammatory activity of standard Diclofenac.

| **Sample Name** | **Concentrations (mg)** | **Absorbance (560nm)** | **% of stabilization** |
|---|---|---|---|
| Diclofenac | 0.5 | 0.092 | 59.18309 ± 0.74 |
| | 0.25 | 0.097 | 57.06305 ± 0.11 |
| | 0.125 | 0.099 | 56.23427 ± 0.26 |
| | 0.0625 | 0.1215 | 46.06892 ± 1.15 |

| | | | |
|---|---|---|---|
| | 0.03125 | 0.129 | 43.03313 ± 0.76 |

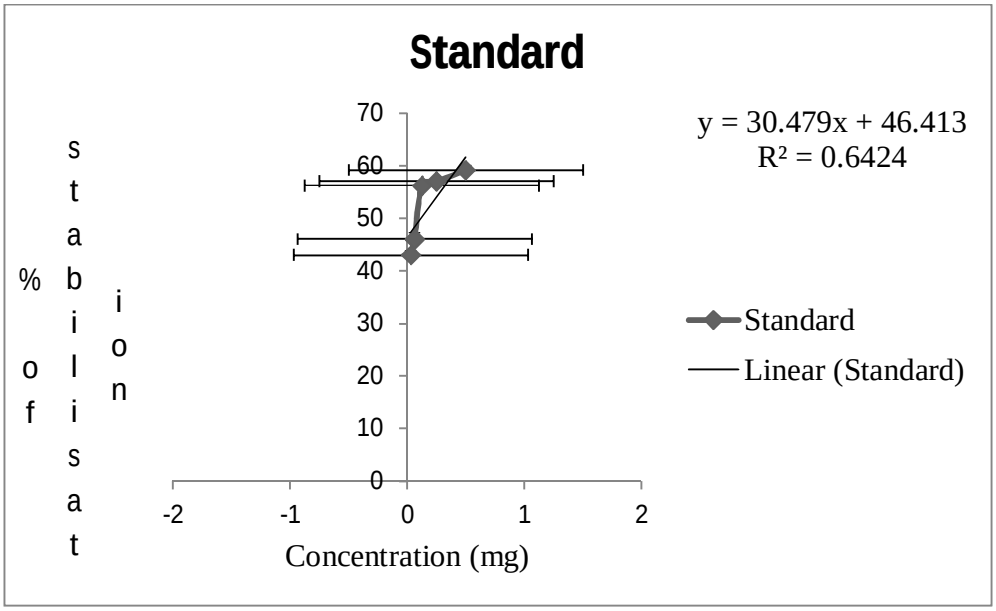



Figure 4.21 Anti-inflammatory activity of Diclofenac

Table 3.7 Anti – inflammatory activity of methanol fraction of shell of *Arachis hypogaea*

| Sample Name | Concentration (mg) | Absorbance (560nm) | % of stabilization |
|---|---|---|---|
| Methanol fraction of shell | 79.2 | 0.1645 | 37.83232 ± 2.84 |
| | 39.6 | 0.162 | 28.05984 ± 1.76 |
| | 19.8 | 0.1475 | 34.14063 ± 4.04 |
| | 9.9 | 0.1675 | 25.75665 ± 0.87 |
| | 4.95 | 0.1785 | 20.66937 ± 0.37 |

Table 3.8Anti-inflammatory activity of dichloromethane fraction of shell of *Arachis hypogaea*

| Sample Name | Concentration (mg) | Absorbance (560nm) | % of stabilization |
|---|---|---|---|
| Dichloromethanefraction of shell | 132.6 | 0.836 | 0 |
| | 66.3 | 0.1435 | 37.54579±53.10 |
| | 33.15 | 0.205 | 26.19048± 37.04 |
| | 16.56 | 0.1515 | 35.89744±50.76 |
| | 8.29 | 0.182 | 11.53846± 16.32 |

Table 3.9Anti – inflammatory activity of xylene fraction of seed of *Arachis hypogaea*

| **Sample Name** | **Concentration (mg)** | **Absorbance (560nm)** | **% of stabilization** |
|---|---|---|---|
| Xylene fraction of shell | 86.4 | 0.05 | 77.06837± 5.49 |
| | 43.2 | 0.5685 | 0 |
| | 21.6 | 0.736 | 0 |
| | 10.8 | 0.246 | 11.53846± 16.31 |
| | 5.4 | 0.37 | 0 |

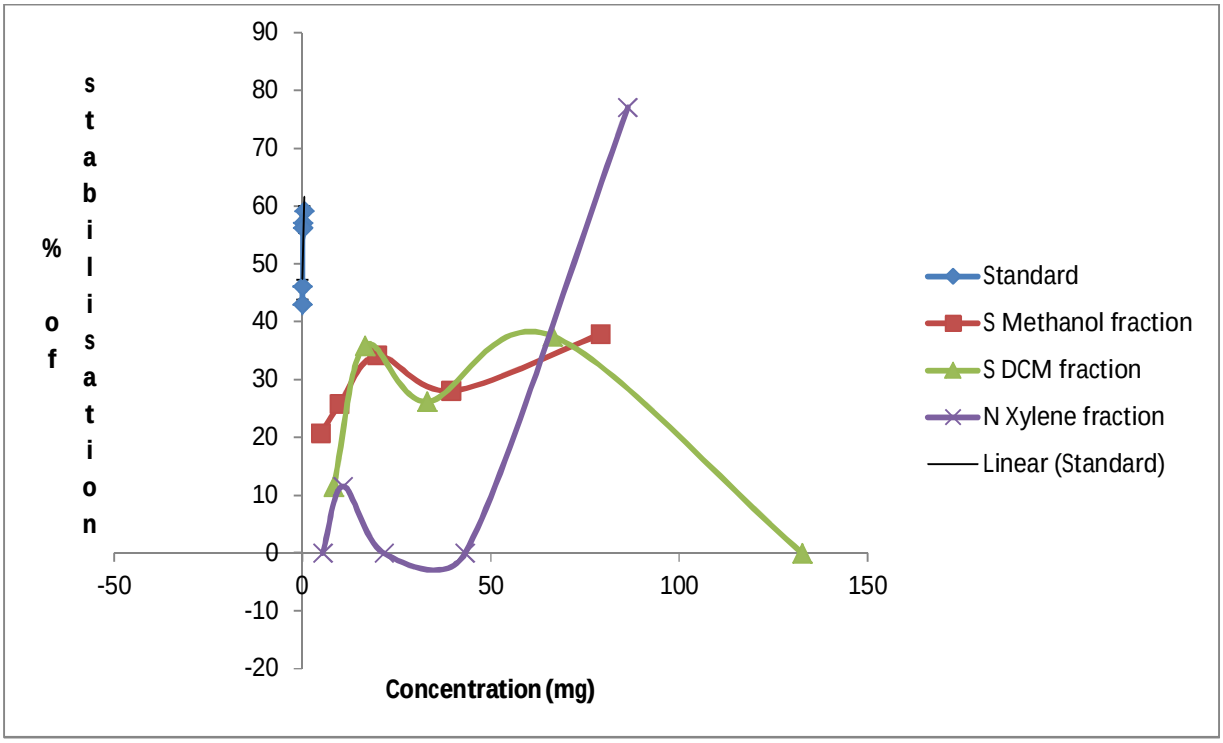



Figure 4.22 % stabilization of RBC of different fractions of seeds and shells of Arachis hypogaea and dichlofenac

**Hemagglutination Inhibition Assay:**

Table 3.10 results of hemagglutination test of different fractions of seeds and shells of *Arachis hypogaea* are given below-

| Concentration (µl) | 50 | 25 | 12.5 | 6.25 | 3.12 | 1.56 | 0.78 | 0.39 | 0.19 | 0.09 | 0.04 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample name | Cell receptor binding | | | | | | | | | | |
| Shell Cyclohexane | - | - | - | - | - | - | - | - | - | - | - |
| Shell Xylene | - | - | - | - | - | - | - | - | - | - | - |
| Shell DCM | - | - | - | - | - | - | - | - | - | - | - |
| Shell Methanol | - | - | - | - | - | - | - | - | - | - | - |
| Nut lower layer pet ether | - | - | - | - | - | - | - | - | - | - | - |
| Nut lower layer Cyclohexane | - | - | - | - | - | - | - | - | - | - | - |
| Nut lower layer DCM | - | - | - | - | - | - | - | - | - | - | - |
| Nut lower layer Xylene | + | - | - | - | - | - | - | - | - | - | - |

Here,

\+ = Agglutinated

\- = Not agglutinated Colum 12 is control

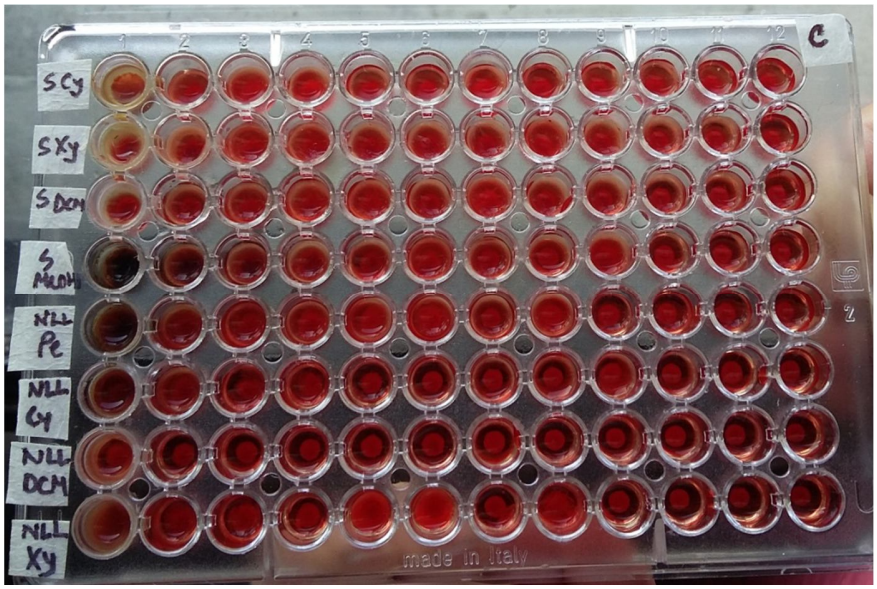



Figure 4.23: Hemagglutination test of different fractions of *Arachis hypogaea*

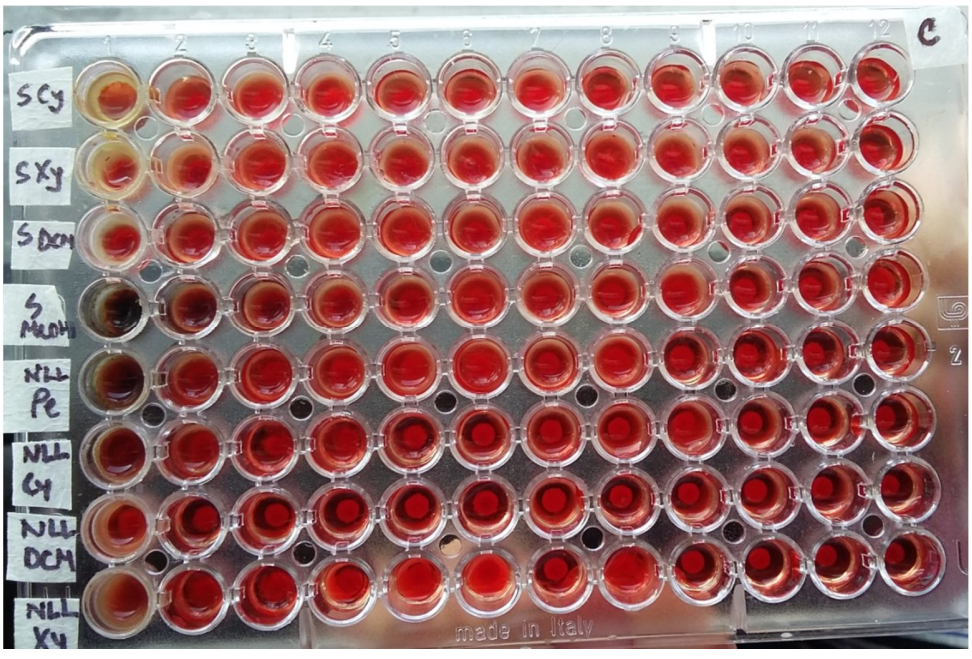


Figure 4.23 Hemagglutination test of different fractions of *Arachis hypogaea*

# UNIT SIX: DISCUSSION

# DISCUSSION

## 1. THIN LAYER CHROMATOGRAPHY

The sky blue, Dip purple, light purple spot present in the plate indicates the presence of valuable compound in the different VLC fraction. The formation of sky blue and purple spot on the plate (VLC fraction) indicates the extensive presence of valuable compounds. From which we can have a preliminary idea of the valuable compounds that may be present in the methanolic extract of *Arachis hypogaea*.

In Case of Polar solvent:
The nut lower layer fractions showed spot of Rf value 1.2, 2.5
The nut middle layer showed spot of Rf value of 3.2
The nut upper layer showed spot of Rf value of 2.1
The shell fractions showed spot of Rf value 1.1, 3.8, 3.9

In case of non polar solvent:
The nut lower layer fractions showed spot of Rf value 2.3
The nut middle layer showed spot of Rf value of 2.1
The nut upper layer showed spot of Rf value of 2.3
The shell fractions showed spot of Rf value 1.3

The primary VLC fractions in semi polar solvent:
The shell fractions showed spot of Rf value 3.7, 3.4, 4.1
The nut lower layer chloroform fractions showed spot of Rf value 2.1
The shell chloroform showed spot of Rf value of 1.9
The nut lower layer DCM fraction showed spot of Rf value of 2.2
The nut lower layer xylene fraction showed spot of Rf value of 1.4

## 2. ANTI BACTERIAL ACTIVITY

The antibacterial activity of the Methanol extract of *Arachis hypogaea* fruit were evaluated by disc diffusion method against gram positive and gram negative bacteria using ciprfloxacillin as

standard. Different VLC fraction of Methanolic extract of *Arachis hypogaea* (pet ether, cyclohexane, xylene, DCM, chloroform and methanol) shows varying degrees of antibacterial activities with zone of inhibition ranging from 7-1.2 mm respectively, while the highest antibacterial activity was seen against with, *Bacillus cereus* ,*Vibrio mimicus* ,*Salmonella typhi and E coli* .

## 3. DPPH TEST

To evaluate the antioxidant activities of different fraction of methanolic extract of the *Arachis hypogae*. DPPH Free Radical Scavenging Assay was used. DPPH reaction has been widely used to test the ability of compounds to act as free-radical scavengers or hydrogen donors and to evaluate the antioxidant activity of foods and plant extracts. DPPH is a stable free radical that can accept an electron of hydrogen radical to become diamagnetic molecule. The reduction in DPPH radical was determined by the decrease of its absorbance at 517 nm (in methanol) induced by antioxidants (Ziying et.al. 2007).

Both nut lower layer and shell fractions of primary VLC fractions showed considerable anti oxidant properties ranging from 86%-45% in varying degrees of the fraction. It should be noted that the nut lower layer fractions showed a higher and better activity compared to the shell fractions but both showed noteworthy activity. The shell fractions showed activity between 62%-45% and the nut lower layer fractions showed activity ranging from 86%-44%

## 4. THIN LAYER CHROMATOGRAPHY OF LECTIN EXTRACT

The sky blue, Dip purple, light purple spot present in the plate indicates the presence of valuable protine compound in the Lectin extract. In the plate of protein the Rf value of $1^{st}$ spot = 0.7,$2^{nd}$ spot = 0.9.$3^{rd}$ spot = 0.97 ,$4^{th}$ spot =0.5 and $5^{th}$ spot=0.2 for shell lectin. In the nut lectin fraction the Rf value of $1^{st}$ spot = 0.3 ,$2^{nd}$ spot = 0.7.$3^{rd}$ spot = 0.8 ,$4^{th}$ spot =0.9.In the plate of carbohydrate test the blue spot present, which Rf value of $1^{st}$ spot =0.9 and $2^{nd}$ spot =0.6 for shell lectin and Rf value of $1^{st}$ spot =0.6, $2^{nd}$ spot =0.7, $3^{rd}$ spot=0.8 .The formation of sky blue, blue and purple spot on the plate indicates the extensive presence of valuable compounds. From

which we can have a preliminary idea of the valuable protein and Carbohydrate compounds that may be present in the lectin extract of *Arachis hypogaea*.

## 5. IN VITRO ANTI-DIABETIC TEST

In the Glucose uptake in Yeast cells method the mechanism of glucose transport across the yeast cell membrane has been receiving attention as in vitro screening method for hypoglycaemic effect of various compounds / medicinal plants. Recent studies on the transport of non metabolizable sugars and certain metabolizable glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers. It is reported that in yeast cells (*Saccharomyces cerevisiae*) glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast is by a facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there is removal of intracellular glucose.

In our result, the nut lower layer fractions showed activity between 85%-54% at highest concentration used. The shell samples showed activity between 78%-55% at the highest concentrations used. As it can be seen that both show note worthy activity but if comparison is to be made the nut lower layer obviously exhibit higher activity compared to the standard

## 6. HEMAGGLUTION INHIBITION INDUCED BY OF *ARACHIS HYPOGAEA* PRIMARY VLC FRACTIONS

Many viruses attach to molecules present on the surface of RBCs. Hemagglutination inhibition assay was performed to investigate the receptor binding affinity of the compounds present in the pet ether, cyclohexane, xylene, dichloromethane, and chloroform and methanol VLC fractions of *Arachis hypogaea* on human erythrocytes. The test was performed with erythrocytes of group O+ and the samples were given at an amount of 4mg/ml with further serial dilution. The different fractions of nut lower layer showed no signs of hemaglutination activity.

# UNIT SEVEN: CONCLUSION

# CONCLUSION

This review had shown that rind of *Arachis hypogaea* possesses numerous bioactivities from natural source which is of better advantage than conventional therapies. The shell and nut lower layer fractions possess greater antioxidant and anti diabetic activity. Its antibacterial property is limited to the shell fractions with poor receptor binding activity

It is surprising to discover that a waste part of the peanut could prove to contain such bioactive molecules that may prove to be useful. Till now the shell was considered a waste part of the nut. Recent studies have revealed its used and an adsorbent or in the waste water treatment.

This thesis report has changed my view about the wonders of nature that lies within each component of a plant.

# UNIT EIGHT: REFERENCE

## REFERNCE


Asibuo, J.Y. Akromah, Richard , Kantanka S., Osei, Dapaah A. ,Kofi H. , Dapaah D., Seth, Agyeman, Adelaide (2008) Chemical composition of groundnut, Arachis hypogaea (L) *African Journal of* Biotechnology, 7(13), 2203-2208 [online] Available at: http://www.academicjournals.org/AJB [Accessed on 13th November 2015]

Anup Sadi (2012) A detailed study of Medicinal plants, *Medicinal plants of Bangladesh* [online] Available at: http://anupsadi.blogspot.com/2012/12/medicinal-plants-of-bangladesh_24.html [Accessed on 29th September 2015]

Asolkar, L.V.; Kakkar, K.K.; Chakre, O.J. (1992). Second Supplement to Glossary of Indian Medicinal Plants with Active Principles. Part I (A-K) (1965-1981), CSIR, New Delhi, India. [online] Available at: http://www.mpbd.info/plants/arachis-hypogea.php [Accessed on 29th September 2015]

Batal A. l, Dale N., Cafe M. (2005) Nutrient Composition of Peanut Meal *Department of Animal Science, School of Veterinary* University of Goia´s, Goiaˆnia, Brazil 74001-970 [online] Available at: http://japr.oxfordjournals.org/content/14/2/254.full.pdf [Accessed on 13th November 2015]

Botanical-online (2015) *Peanut Properties* [online] Available at: http://www.botani cal-online.com/english/peanutproperties.htm# [accessed on 13th November 2015]

Cannell RJP. (1998). How to approach the isolation of a natural product, in Natural Products Isolation. 1st ed. (Cannell, R. J. P., ed.), Humana Press, New Jersey, pp. 1-51.

ENCYCLOPEDIA BRITANICA (2015) *Fabaceae* [online] Available at: http://global.britannica.com /plant/Fabaceae [Accessed on 3rd November 2015]

Ghani A. (1998) *Present State-Of-The Art Of Traditional Medicine Practice In Bangladesh* Jahangirnagar University [online] Available at: http://www.itmrc.org/publication/ch_5.htm [Accessed on 1st Nivember 2015]

Hemagglutination Assay (HA) and Hemagglutination Inhibition Assay (HI): AI and NDV. (2006)[online] p.3. Available at: http://www.cfsph.iastate.edu/HPAI/resources/SOPs/HA_HI.pdf [Accessed 6 Nov. 2015].

Hou Y., Hou Y., Yanyan L., Qin G., Li J. (2010). Extraction and purification of a lectin from red kidney bean and preliminary immune function studies of the lectin and four Chinese herbal polysaccharides. .*J Biomed Biotechnol;* 9:217-342.

Ingale S., Srivastava S.K, (2011) Nutritional study of new variety of groundnut (Arachis hypogaea L.) *JL-24* seeds, *African Journal of Food Science* 5(8), pp. 490 – 498 [online] Available at: http://www.acade mic journals.org/journal/AJFS/article-full-text-pdf/594849A9689 [Accessed on 3rd November 2015]

Iman A.,Farag A.,Ahmed A., Zahran (2014) Groundnut (Arachis hypogaea L.) growth and yield responses to seed irradiation and mineral fertilization, *IOSR Journal of Agriculture and Veterinary Science*, 7(5-3),63-70 [online] Available at: http://iosrjournals.org/iosr-javs/papers/vol7-issue5/Version-3/J07536370.pdf [Accesseed on 13th November 2015]

Jaishankar, M. , Mathew, B. B. , Shah, M. S. , T.P., K. M. , & K.R., S. G. (2014) Biosorption of Few Heavy Metal Ions Using Agricultural Wastes. *Journal of Environment Pollution and Human Health* 2(1), 1-6 [online] Available at: http://pubs.sciepub.co m/jephh/2/1/1/# [Accessed on 20th November 2015]

Kar, B., Kumar, R., Karmakar, I., Dolai, N., Bala, A., Mazumder, U. and Haldar, P. (2012).Antioxidant and in vitro anti-inflammatory activities of Mimusopselengi leaves. *Asian Pacific Journal of Tropical Biomedicine,* 1:5978.

Lewis, J. D. (2012). *15.3b Natural Product Screening- Anti-oxidant screen of extract Crude Extract*, 2nd ed. [ebook] p.1. Available at: https://courses.cns.utexas.edu/.../15.3b-Natural-Product-Screening-Anti-oxidant Properties [Accessed on 20th Jul. 2015].

Lewis W.E., Harris G.K. , Timothy H.s., Brittany L.W. , Lisa L.D. (2013) Antioxidant and Anti-Inflammatory Effects of Peanut Skin Extracts, Food and Nutrition Sciences 4:22-32 [online] Available at: https://www.google.com.bd/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwjnJiA77rJAhXQno4KHcjCCM0QFggfMAA&url=http%3A%2F%2Fwww.scirp.org%2Fjournal%2FPaperDownload.aspx%3FpaperID%3D35240&usg=AFQjCNGYFlmd53D6Yfm_a5cIXoz1x0ZXuA&sig2=dOGieB43_MvAZFRwm6bfCA&bvm=bv.108194040,d.c2E [Accessed on 1st December 2015]

Mandloi S.,Radadia B.B., Visavadia M., Vaghela A. (2014) A Review Of Chemical Characteristics (acid Value And Iodine Value) Of Peanut Oil, *Weekly Science Research Journal* 1(30) [online] Available at: http://www.weeklyscience.org/UploadedArticle/69.pdf [Accessed on 1st December 2015]

MedicinalPlants (no date) *Arachis Ahpogaea L* [online] Available at: http://www.mpbd.info/plants/arachis-hypogea.php [Accessed on 29th September 2015]

Mishra, A., Kavrekar, V. and S.Nair, S. (2013).Evaluation of In Vitro Anti diabetic Activity of Selected Plant Extracts, .*International Journal of Pharmaceutical Science Invention*, 12(4), pp.12-19.

Newman DJ and Cragg GM. (2007). Natural products as sources of new drugs over the last 25 years. *J Nat Prod*. 70: 461-477.

Orgchem.colorado.edu, (2015). *Thin Layer Chromatography (TLC)*. [online] Available at: http://orgchem.colorado.edu/Technique/Procedures/TLC/TLC.html [Accessed 20 Jul. 2015].

Rahman DMA. (2007) Conservation of Medicinal Plants in Bangladesh. Forestry and Wood Technology Discipline, Khulna University,Khulna.

Rahman, A.H.M. Mahbubur (2013) Ethno-botanical Survey of Traditional Medicine Practice for the Treatment of Cough, Diabetes, Diarrhea, Dysentery and Fever of Santals at Abdullahpur Village under Akkelpur Upazilla of Joypurhat District, Bangladesh, *Biomedicine and*

*Biotechnology* 1(2): 27-30 [online] Available at: http://pubs.sciepub.com/bb/1/2/6/# [Accessed on 1st November 2015]

Ross E. (2010) Review Health Benefits of Nut Consumption, *Nutrients* [online] Available at: www.mdpi.com/journal/nutrients [Accessed on 1st December 2015]

Sadi A. (2012) Prankakoli, *Medicinal Plants of Bangladesh* [online] Available at: http://anupsadi.blogspot.com/2012/12/medicinal-plants-of-bangladesh_24.html [Accessed on 3rd November 2015]

Sim. W. E., Lai. Y. S, Chang. P. Y, (2012), Antioxidant capacity, nutritional and phytochemical content of peanut (Arachis hypogaea L.) shells and roots, *African Journal of Biotechnology* 11(53) [online] available at: http://www.academicjournals.org/article/article1380813278_Sim%20et%20al.pdf [Accessed on 29th September 2015]

SobolevV.S., Khan I.S., Tabanca N.,Wedge D.E..,Manly S.P.,Cutle J.S., Coy M.R., Becnel J.J, Neff S.A., Gloer J.B. (2011) Biological Activity of Peanut (Arachis hypogaea) Phytoalexins and Selected Natural and Synthetic Stilbenoids, *Journal Of Agricultural And Food Chemistry*, 59:1673-1682 [online] Available at: http://www.afpmb.org/sites/default/files/pubs/dwfp/publications/FY11/2011%20Peanut-JAFC.pdf [Accessed on 1st December 2015]

USDA PLANTS (2015) *Arachis hypogea L.* [online] Available at: http://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=26463 [Accessed on 1st November 2015]

USDA (2015) National Nutrient Database for Standard Reference Release 2 *United States Department of Agricultural Research Service* [online] Available at: http://ndb.nal.usda.gov/ndb/foods/show/4808?fg=&man=&lfacet=&count=&max=&sort=&qlookup=&offset=&format=Full&new=&measureby= [Accessed on 10th November 2015]

Pankaj, k. (2015).Methods for Rapid Virus Identification and Quantification.*Labome* [online] Available at: http://www.labome.com/method/Methods-for-Rapid-Virus-Identification-and-Quantification.html [Accessed 6 Nov. 2015].

Saha R.K., Acharya S., JamiruddinM. ,RoyP. , IslamS.M.,Shovon S.S.H. .(2014). Antimicrobial effects of a crude plant lectin isolated from the stem of Tinospora tomentosa. *The Journal of Phytopharmacology*; 3 (1), 44-51.