NUTRITIONAL,CHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SEEDS AND SEED EXTRACTS

A PROJECT REPORT

Submitted by

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BONAFIDE CERTIFICATE

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(INTERNAL EXAMINER) **(EXTERNAL EXAMINER)**

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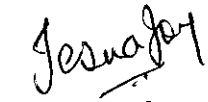

[Jesna Joy]

ABSTRACT

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An attempt was made to study the composition of seeds and to investigate on the antioxidant and antimicrobial capabilities of seeds and its extracts. Four seed samples (Abelmoshchus esculentus, Solanum lycopersicum, Moringa oleifera, Arachis hypogaea) were the for the above mentioned objectives. The methanolic and acetone solvent extracts were prepared. The acetone extracts of samples were tested for their antimicrobial activity against six bacteria using Agar well difussion. Extracts of Tomato seeds showed positive results for Salmonella typhi,Escherishia coli and Bacillus subtilis.The acetone extracts of Drumstick seeds showed positive results for Staphylococcus aureus and Bacillus subtilis.Ladies finger acetone extract showed a low activity on Staphylococuss aureus.The composition of all four seed samples were tested using AOAC methods and fibre,protein,fat,ash, carbohydrate,moisture energy were calculated along with the trace elements Calcium,Magnesium and Iron.The antioxidant activity was studies using two invitro assays(Total Antioxidant and DPPH radicle scavenging assay). Perentage of DPPH radicles by the methanolic extract of Abelmoshchus esculentus,Solanum lycopersicum,,Moringa oleifera and Arachis hypogaea increases with increase in concentration.

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INTRODUCTION

1. INTRODUCTION

A seed is a small embryonic plant enclosed in a covering called the seed coat(testa), usually with some stored food. It is the product of the ripened ovule of gymnosperm and angiosperm plants which occurs after fertilization and some growth within the mother plant. The formation of the seed completes the process of reproduction in seed plants, with the embryo developed from the zygote and the seed coat from the integuments of the ovule. Seeds protect and nourish the embryo or baby.

Many seeds are edible. Seeds also provide most cooking oils, many beverages and spices and some important food additives. Seeds are used to propagate many crops such as cereals, legumes, forest trees, turfgrasses and pasture grasses. Some seeds are also poisonous. The world's most important clothing fiber grows attached to cotton seed. Many important nonfood oils are extracted from seeds. Seeds are the source of some medicines including castor oil, tea tree oil and the discredited cancer drug, Laetrile.Many seeds have been used as beads in necklaces and rosaries including Job's tears, Chinaberry and rosary pea. Other seed uses include: Seeds once used as weights for balances

SEED OILS

Although many different parts of plants may yield oil, in commercial practice, oil is extracted primarily from seeds. Vegetable fats and oils are lipid

materials derived from plants. Physically, oils are liquid at room temperature, and fats are solid. Chemically, both fats and oils are composed of triglycerides, as contrasted with waxes which lack glycerin in their structure. Although many different parts of plants may yield oil, in commercial practice, oil is extracted primarily from seeds.

Vegetable fats and oils may be edible or inedible. Examples of inedible vegetable fats and oils include processed linseed oil, tung oil, and castor oil used in lubricants, paints, cosmetics, pharmaceuticals, and other industrial purposes.

Seed oils are important sources of nutritional oils.It is known in the art that seed oils, that is oils derived from plants by distillation, expression or extraction may have antimicrobial activity when exposed to bacterial cells. Many important non food oils are extracted from seeds. Linseed oil is used in paints. Oil from jojoba and crambe are similar to whale oil.Seeds are the source of some medicines including castor oil, tea tree oil and the discredited cancer drug.The therapeutic properties of carrot seed oil are antiseptic, carminative, cytophylactic, depurative, diuretic, emmenagogue, hepatic, stimulant, tonic and vermifuge.The medicinal properties of seed oil of Pongamia glabra are well known in traditional Indian medicine. It has antimicrobial activity against several organisms.

LIST OF SEEDS:

Abelmoshchus esculentus (Ladies finger) seed

Solanum lycopersicum (Tomato) seed

Arachis hypogaea (Groundnut) seed

Moringa oleifera (Drumstick) seed

***Abelmoshchus esculentus* (Ladies finger)**

Okra , known by many other names, is a flowering plant in the mallow family (along with such species as cotton, cocoa, and hibiscus), valued for its edible green fruits.*Abelmoschus esculentus* is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits or pods containing round, white seeds. The fruit is a capsule up to 18 cm long, containing numerous seeds. Okra seeds may be roasted and ground to form a non-caffeinated substitute for coffee.*Abelmoschus esculentus* is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits or pods containing round, white seeds. The fruits are harvested when immature and eaten as a vegetable.

A traditional food plant in Africa, this vegetable has potential to improve nutrition, boost food security, foster rural development and support sustainable landcare. Okra oil is a pressed seed oil, extracted from the seeds of the okra. The greenish yellow edible oil has a pleasant taste and odor, and is high in unsaturated fats such as oleic acid and linoleic acid (Franklin W. Martin et al.,1982). The oil content of the seed is quite high at about 40%. Oil yields from okra crops are also high. At 794 kg/ha, the yield was exceeded

only by that of sunflower oil in one trial, (Buchanan, *et al.*, 1990).Unspecified parts of the plant reportedly possess diuretic properties.(Felter *et al.*,2007) (Moench,2004)

***Solanum lycopersicum* (Tomato)**

The tomato (*Solanum lycopersicum*) is a herbaceous, usually sprawling plant in the nightshade family that is typically cultivated for its edible fruit.Tomato seeds are occasionally organically produced as well, but only a small percentage of organic crop area is grown with organic seed.

An edible oil is obtained from the seed (Uphof. J. C.,1959) (Usher. G,1974)(Hill. A. F.,1952). Suitable for culinary purposes (Facciola. S.,1990). The seed is small and it would be very fiddly to utilize. It is only viable to use the seed as a source of oil if large quantities of the plants are being grown for their fruits and the seed is not wanted. A semi-drying oil is obtained from the seed. It can be used in making soap.The alcoholic extract of tomato possesses CNS depressant and analgesic properties.

***Moringa oleifera* (Drumstick)**

Moringa oleifera, commonly referred to simply as "Moringa" is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. It is an exceptionally nutritious vegetable tree with a variety of potential uses. The seeds from this plant contain active coagulating agents characterized as dimeric cationic proteins, having molecular weight of 13 kDa and an isoelectric point between 10 and 11. The

seeds also have antimicrobial activity and are utilized for waste water treatment. In some developing countries, the powdered seeds of *M. oleifera* are traditionally utilized as a natural coagulant for water purification because of their strong coagulating properties for sedimentation of suspended undesired particles (Kalogo *et al.*,2000; Anwar *et al.*, 2007*)*.

The Moringa seeds yield 38–40% edible oil (called ben oil from the high concentration of behenic acid contained in the oil). The refined oil is clear, odorless, and resists rancidity at least as well as any other botanical oil. The seed cake remaining after oil extraction may be used as a fertilizer or as a flocculent to purify water. The bark, sap, roots, leaves, seeds, oil, and flowers are used in traditional medicine in several countries.

Arachis hypogaea (Groundnut)

The peanut, or groundnut (*Arachis hypogaea*), is a species in the legume "bean" family (Fabaceae) native to South America, Mexico and Central America. Peanuts are known by many local names, including earthnuts, ground nuts, goober peas, monkey nuts, pygmy nuts and pig nuts. The term "Monkey nut" is often used to mean the entire pod. Peanuts can be used like other legumes and grains to make a lactose-free milk-like beverage, Peanut milk. Low grade or culled peanuts not suitable for the edible market are used in the production of peanut oil. The protein cake (oilcake meal) residue from oil processing is used as an animal feed and as a soil fertilizer. Low grade peanuts are also widely sold as a garden bird feed.

Peanut oil is often used in cooking, because it has a mild flavor and its relatively high cooking temperature. Its high monounsaturated content makes it heart-healthy and resistant to rancidity. There are several types of peanut oil including: aromatic roasted peanut oil, refined peanut oil, extra virgin or cold pressed peanut oil and peanut extract. Studies have shown that refined peanut oil is safe for peanut allergic individuals because the protein is destroyed during the processing. Paint, varnish, lubricating oil, leather dressings, furniture polish, insecticides, and nitroglycerin are made from peanut oil.

OBJECTIVES:

The present study is formulated to study the composition and effectiveness of seeds and seed oil extracts to meet increasing consumer demands

1) To evaluate quantitatively Nutritional and Chemical constituents present in the seeds
2) To investigate Antimicrobial Activity of seed oils and crude extracts
3) To understand Antioxidant properties of the seed extracts

LITERATURE REVIEW

LITERATURE REVIEW

2.1 IMPORTANCE OF SEEDS:

Seeds account for 70 percent of food consumed by humans, and are also the major feeds for domestic animals. Their importance cannot be overstated. World seed production is dominated by the cereals, and even the production of wheat, maize, or rice alone by far exceeds that of all the other crops. As a concentrated source of carbohydrate, cereals provide for the human diet, livestock feed, and industrial raw materials. They are also an important source of protein, oil, vitamins, and fiber. Grain legumes , particularly soybeans and groundnuts (peanuts) are an important source of proteins and vegetable oils, which are used in margarine and cooking fats, and have applications in paints, varnishes, and plastics, as well as the manufacture of soaps and detergents. An understanding of seeds is therefore an essential prelude to human attempts to improve their quality and yield, whether it be by conventional breeding techniques or the novel approach of genetic engineering. (Bewley, J. Derek.. 2010)

2.2. PROXIMATE ANALYSIS:

2.2.1 Nutritional and Chemical Importance of Seeds:

Seeds contain all the important nutrients needed for human growth. They are excellent sources of protein and the essential unsaturated fatty acids which are necessary for health. They are also one of the best natural sources of lecithin, most of the B-complex vitamins and vitamin E, which are perhaps the most important

elements for the preservation of health and prevention of premature ageing. Besides, they are rich sources of minerals and supply the necessary bulk in the diet.

Seed oils are important sources of nutritional oils, industrial and pharmaceutical importance.The characteristics of oils from different sources depend mainly on their compositions and no oil from a single source can be suitable for all purposes [Mohammed and Jorg, 2003].

Seeds contain pacifarins, an antibiotic resistance factor, which increases man's natural resistance to disease. They also contain auxones, natural substances which help produce vitamins in the body and play an important role in the rejuvenation of cells, thereby preventing premature ageing. Sprouted seeds are excellent live nourishment. They are valuable sources of protein, vitamins, and complex carbohydrates. Germinated seedsenormously increase their nutritional value. All seeds should ideally be eaten raw but those, which can be sprouted, should be consumed in that form to derive maximum nutrition.

2.2.2 Other Nutritional and Chemically Important seeds:

Annona muricata (Custard Apple) seed is a good source rich in minerals and oil. The oil extracts exhibited good physicochemical properties and could be useful for cosmetic and other industrial applications. (A. Kimbonguila , J.M. Nzikou et al., 2010) *Hura Crepitans* seed could act as protein supplement, though its suitability as food supplement depends on factors like the presence of antinutritional factors and the digestibility of its nutrients.(*Oderinde et al.,*

*2009)*Physicochemical constants of *Momordica charantia* Linn.studied were helpful to identify the quality of oil and oil products for possible industrial or commercial uses. Karela seed oil is comparable to other oils and can be utilised in the paint, varnish, ink industries and also recommended for possible human consumption after proper refining. *(M. Abbas Ali., et al.2009)*

2.2.3 Uses of Selected Samples along with their Phytoconstituents:

a) ***Moringa oleifera***:

The coagulant of seeds of *M. oleifera* could be used for wastewater treatment. The seeds and gum are widely used in India folk medicine.The seed oil of *M. oleifera* is also used as a lubricant for fine machinery, such as timepieces, for its little tendency of deteriorating and becoming sticky. Moreover, the oil has the capacity to absorb and retain volatile substance and is therefore valuable in the perfume industry (Foidl et al., 2001).

2.3 ANTIMICROBIAL ACTIVITY

2.3.1 Antimicrobial Activity of Seed oils and Extracts:

It is known in the art that plant essential oils, that is oils derived from plants by distillation, expression or extraction may have antimicrobial activity when exposed to bacterial cells. Consumer acceptance of these essential oils is high because they usually have the fragrance of the plant from which they were extracted. When used for animal use, the animals do not commonly shy away from them because again,

they have the odor of plants from which they were derived, and such odors are not unfamiliar to many animals.Essential oils mixed with carriers have a lot of potential veterinary and human uses. For example in the veterinary world they may be used as teat dips, or disinfecting topicals for skin ulcers, for shampoos, for topical gels and creams, for anti-fungals, and even to be taken internally for use in the GI tract.There is a continuing need for increasing the cellular uptake of plant essential oils in order to enhance their anti-bacterial effect. Some researchers have theorized that plant essential oils soften the walls of the bacteria then permeate them thus causing the enhanced anti-bacterial effect. (Vaara, "Agents That Increase the Permeability of the Outer Membrane", M*icrobiological Reviews,* September 1992, Vol. 56(3)

2.3.2 Other Potential Seed Oils Exhibiting Antimicrobial Activity:

Black pepper was found to be effective against *Salmonella* and *Bacillus subtilis*.(Ifra Ghori et al.,2009) Similar report have been by (Reddy *et al.*, 2001) about antibacterial activity of the pure isolates from black pepper against *Bacillus subtilis* . However, further research is needed to optimize the effective use of this agent in clinical practice (Molan, 2001).

Nigella sativa seed oil possesses antimicrobial activity against several multidrug resistantpathogenic bacteria and may be used topically in susceptible cases. The antimicrobial activity of this oil may be attributed to the presence of thymoquinone, thymohydroquinone and thymol in the oil all of which possessed antimicrobial activity.(MT Salman et al.,2008)

The results of antimicrobial activity of Momordica charantia seed oil indicated that S. aureus was the most sensitive microorganism tested, while low inhibitory activities were evidenced against strains of E. coli and C. albicans . (Alessandra Braca et al.,2008)

2.4 ANTIOXIDANT ACTIVITY

2.4.1 Importance of Antioxidants in Seed Oil

The shelf life of vegetable oils in food uses and their applicability in industrial situations is greatly dependent on their oxidative stabilities. Methods of improving oxidative stability values currently available include genetic modifications, compositional changes via chemical means, as well as the inhibition of oxidation by means of substances known as antioxidants.Whether applied for industrial applications, one of the major challenges in the utilization of the more environmentally friendly vegetable oils for industrial applications is their poor oxidative stability (Honary, 2004), (Howell, 2007).

Numerous experimental works have established the positive effect of anti-oxidants on the oxidative stability of vegetable oils for both edible uses and industrial uses. An important class of anti-oxidants consists of the phenolic compounds Butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), Propyl Gallate, and Tert-butyl Hydroquinone (TBHQ). Their use in vegetable oils meant for domestic and industrial processes is widespread. Vegetable oils in their natural form possess constituentsthat function as natural antioxidants. Amongst them are ascorbic acids,-tocopherole, -carotene, chlorogenic acids and flavanols (Ullah et al., 2003).

2.4.2 Other Seed Oils With Effective Antioxidant Activity

Tests conducted to investigate the effectiveness of natural anti-oxidants contained in red pepper oil added to soybean and sunflower oils indicate that they provide variable protection against light induced auto-oxidation. Measuring fatty acid formation and the measurement of peroxide values as a means of monitoring oxidation,results indicate an inhibitive effect on oxidation (Ullah et al., 2003).

In another study which monitored the inhibitive action of tocopherols on rapeseed and palm kernel oils by measuring the presence of the oxidation product, monoaldehyde, indicate some measure of protection provided by these natural anti-oxidants (Emanuel et al., 1967).

2.4.3 Antioxidant Work In the Selected Seed Sample:

Polyphenolic profile of okra (*Abelmoschus esculentus*), was identified and quantified. Since the knowledge about the okra polyphenolic compounds is limited, the seeds and the skins of okra were separately analyzed. The seeds, which represent the 17% of the vegetable and were found to be richer in phenolic compounds.These findings in associations with the high content of okra in carbohydrates and proteins enhance the importance of this foodstuff in the human diet. **(Panagiotis Arapitsas et al.,2008)**

Moringa oleifera leaf extracts were tested in two stages of maturity using standard *in vitro* models. The successive aqueous extract of *Moringa*

oleifera exhibited strong scavenging effect on DPPH free radical, superoxide, nitric oxide radical and inhibition of lipid per oxidation. The data obtained suggested that the extracts of *Moringa oleifera* both mature and tender leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage. (S. Sreelatha et al.,2009)

The antioxidant activity and identification of the antioxidant component of peanut seed testa were investigated. The antioxidant activity of peanut seed testa was studied in the linoleic acid model system by using the ferric thiocyanate method. Among the five organic solvent extracts, the ethanolic extracts of peanut seed testa produced higher yields and stronger antioxidant activity than other organic solvent extracts. (W.J Yen et al.,2005)

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Collection of different varieties of plant seed samples:

Different types of seasonal plant seeds based on their medicinal properties were collected from markets of Tamil Nadu during January 2010.

Table 3.1 Seed Sample and Location Collected

Seed Samples	Arachis hypogaea Groundnut)	Moringa oleifera (Drumstick)	Solanum lycopersicum (Tomato)	Abelmoshchus esculentus (Ladies finger)
Location of Collection	Oil Mill	Super Seeds, R.S.Puram	Super Seeds, R.S.Puram	Super Seeds, R.S.Puram

3.2 Seed Sample Processing:

Healthy seeds(without weeds, other seeds, inert materials like insects, fungal bodies) were analysed.The samples were washed, dried and set for grinding.

3.3 Grinding:

The seeds were ground in between fine and coarse size.

3.4 Oil Extraction from Seed Sample:

The appropriately ground seed samples were subjected to solvent extraction. Fractionized solvents were added in the following order (Hexane, Chloroform, Acetone, Methanol-based on increasing polarity) and kept for an extraction time of (16h to 24h).

3.5 Extract Filtering:

The extract was filtered to separate it from the crushed seed

3.6 Extract Concentration:

The solvent extracts were subjected to natural evaporation for few days until it was concentrated

3.7 Crude Extract Storage:

The concentrated crude was collected and stored in clean amber bottles.

3.8 Proximate Analysis

3.8.1 Glassware and chemicals

Good quality glassware and chemicals were used for all the tests. All the glassware were of brand Borosil or Corning. They were washed with good detergent, rinsed in tap water and socked in chromic acid clearing solution.

3.8.2 Clearing solution (Mahadevan & Sridar, 1996)

Potassium dichromate	- 60 g
Con H2SO4	- 60 ml
Dist. Water	- 1 L

Potassium dichromate was dissolved in warm water, cooled and sulphuric acid was added slowly. It was mixed thoroughly and used for cleaning glassware. Then they were rinsed thrice in tap water, finally rinsed in distilled water and dried in hot air oven. Dried glassware and media were sterilized in an autoclave for 15 min at 15 lb/sq inch pressure. These sterilization and cleaning methods were used for further experiments.

3.8.3 Chemicals

Analytical grade chemicals supplied by Loba, Hi-Media, S.D. Fine Chemicals, E.Qualigens and Sigma Chemicals (USA) were used in this study.

3.8.4 Plant collection and identification

The plant specimens were collected at a flowering stage and identified as *Ipomoeanauritiana.* (Fig 2.1) with the help of Flora of Madras Presidency (Gamble, 1967) and the flora of Tamilnadu and Carnatic (Mathew, 1991). The plant *I.mauritiana* was collected from atakatti near Kerala, India and authenticated by Prof. N. Raaman Director, Center for Herbal Sciences, Center for advanced studies in Botany, University of Madras and Chennai, India. When the plants were at flowering stage, the floral characters were used to identification of the plant with the help of floras (Gamble, 1967). These organs were photographed in closer views with the help of Nikon digital camera. The plant tuber part was collected after a through investigation to check for any pathological disorders and from contamination from other plants, washed with distilled water and shade dried.

3.8.5 Preparation of extracts

A total of 5 kg of powder of plant aerial parts were separated into 2 kg and 3 kg. The 2 kg powder was soked at room temperature in methanol for 48 hr. The extract was suctioned filtered using Whatmann filter paper. This was repeated for 2 to 3 times and similar extracts were pooled together and concentrated at 40C to 45C under reduced pressure using vaccum rotary

evaporator type 350. The remaining 3kg of plant powder was soaked separately at room temperature in hexane. The residual plant material was extracted successfully by fractionized with increasing polarity chloroform and methanol in the same manner as followed for hexane (Harborne, 1998; Raaman, 2006). The concentrated chloroform, hexane and methanol crude extracts were subjected to column chromatography to isolate to active principles.

3.8.6 Determination of nutritive values

Since the plant used under study is used as staple food by some people, it was decided to determine the nutrient values of the tuber. The amount of carbohydrate, protein, fat, fibre, some minerals and few vitamins were determined per 100 g of dry powder of the tuber. The protein values were calculated from the nitrogen content and the factor used was 6.25. The fat contents were obtained from total ether extractives. The carbohydrate content was the difference between 100 and the sum of of moisture, protein, fat, fibre and ash contents. The moisture, fibre and fat contents were calculated as per standard procedures given in IS 1990. The food energy was calculated from the content of the proximate principles assuming that proteins, carbohydrates and fats yield 4,4 and 9K cals respectievely per gram.

The mineral elements were estimated by standard procedures. Calcium and magnesium were estimated. The elements were estimated with the help of Atomic Abosorbic Spectrophotometry. Four vitamins were also quantified. Vitamin A, Vitamin B (Thiamine), Vitamin B2 (Riboflavin) and Vitamin C

(Ascorbic acid) were estimated). It is usual practice to express Vitamin A value of foodstuff in terms of international units. In vegetable food, the carotene content is usually given assuming that 0.6 g of carotene is equivalent to one I.U. of Vitamin A. All the values are given per 100 g of edible portion of the tuber.

3.8.7 Determination of crude protein (AOAC, 1990)

Principle

Sample was digested in H_2SO_4 using $CuSO_4$ as catalyst, converting nitrogen to ammonia which was distilled and titrated.

Apparatus

Kjeldahl flask : 500-800 ml capacity

Distillation assmenbly- The assembly consisted of a round bottomed flask of 1000 ml capacity fitted with a rubber stopper through one end of the connecting bulb tube. The other end of the bulb was connected to the condenser which was attached by the means of a rubber tube to a dip tube which dips into a known quality of standard sulphuric acid solution contained in a conical flask of 5000 ml capacity, to which 3 or 4 drops of methyl red indicator solution was added.

Reagents

1. Sodium sulphate anhydrous

2. Copper sulphate

3. Concentrated sulphuric acid

4. Sodium hydroixide solution- 500g of sodium hydroxide was dissolved in 1000 ml water

5. Standard sulphuric acid- 0.5 N

6. Standard sodiuim hydroxide solution- 0.1 N

7. Methyl red indicator solution- 1 g of methyl red indicator was dissolved in 100 ml of methanol

Procedure

One gram of the powdered sample was transferred to kjeldahl flask and 7.0 g of sodium sulphate anhydrous, 0.7 g of copper sulphate and 25 ml of sulphuric acid were added. The flask placed in an inclined position was heated below the boiling point of the acid until the frothing ceased. The heat was increased until the acid boiled vigorously (for 3 H and 30 min). Water (200 ml) was added to kjeldahl flask and after cooling the contents of the flask, 50% sodium hydroxide solution was added by the side of the flask so that it did not mix at once with the acid solution but forms a layer below the acid layer. The apparatus was assembled taking care to see that the tip of the tube extended below the surface of the 20 ml of standard sulphuric acid solution (0.5 N) in the receiver. Three drops of methyl red indicator solution was added to the acid. The contents of the flask were mixed by shaking and distillation was done till all the ammonia had passed over into standard sulphuric

acid solution till the color in the receiver turn from red to yellow. Blank determination using all reagents in the same quantities but without the material tested was carried out.

Calculation

On an average, most proteins have 16% nitrogen in their compostion. In the other words, 1 mg of nitrogen equals to 6.25 mg of proteins. Based on stoichiometric relationships involved in titrations, 1 ml of 0.01 N HCL is equivalent to 140 mf of nitrogen present in ammonia. Thus, from the volume of standard HCL used for titration, the amount of nitrogen in the sample were calculated and the value multipled by 6.25.

$$\text{Percentage of protein} = \frac{(\text{Blank titre value} - \text{Sample titre value}) \times \text{Strength of 0.1 N NaOH} \times 1.40007 \times 6.25}{\text{Weight of the sample}}$$

Where

6.25 = nitrogen factor

1.4007 = equal to protein in Iysine Hydrochloric acid.

3.8.8 Determination of crude fat (IS, 1990)

Reagents

Petroleum ether of boiling range 40C to 60 C

Hexane food grade confirming to IS: 3470-1966

Procedure

The prepared sample (5 g) was dried in hot air oven at 105+_ 2C for atleast 2 hr and was extracted with petroleum ether or hexane in Soxhlet extractor. The extraction was done at condensation rate of 5 to 6 drops per sec for 4 hr and 2 to 3 drops per sec for 16 hr. the extract was dried on steam bath for 30 min cooled in desiccators and weighed. Alternate drying and weighing were done at 30 min intervals till the difference between the two successive weighing was less than 1 mg and the lowest mass was noted.

Calculation

$$\text{Percentage of crude fat (On Moisture free basis)} = \frac{100 \times (M1-M2)}{M}$$

Where

M1 = Mass in g of the extraction flask with dried extract

M2 = Mass in g of the extraction flask

M = mass in g of the dried sample taken for the test

3.8.9 Determination of the crude fibre (IS, 1990)

Reagents

Sulphuric acid – 0.255 N (1.25% v/v), accurately prepared

Sodium hydroxide solution – 0.313 N (1.25% m/v), accurately prepared

Procedure

The dried material (2 g) was extracted from the fat content with petroleum ether or hexane using in Soxhlet extractor (alternatively, the residue from the crude fat determination can be used). The fat free dry residue was transferred to 1 L conical flask. Boiling dilute sulphuric acid (200 ml) was added to the flask with the fat free material and immediately the flask was connected with reflux condenser and heated for 30 min. The contents were filtered through fine linen held in a funnel. The residue was washed with boiling water. The residue on the linen was

washed into 200 ml of boiling sodium hydroxide solution. The solution filtered, washed with boiling water and transferred to a Gooch crucible prepared with a thin but compact layer of ignited asbestos. The residue was washed with hot water and then with 15 ml of 95% (by volume) ethyl alcohol. The Grooch crucible and the contents were dried at 105 C in the hot air oven to constant mass. It was cooled and weighed. The contents of the Gooch crucible were incinerated at 600 C in a muffle furnace until all the carbonaceous matter was burnt. The crucible containing the ash was cooled in desiccators and weighed.

Calculation

$$\text{Percentage of crude fat (on moisture free basis)} = \frac{100 \times (M1 - M2)}{M}$$

Where

M1 = Mass in g of Gooch crucible and contents before ashing

M2 = Mass in g of Gooch crucible containing asbestos and ash

M = Mass in g of the dried sample taken from test

3.8.10 Estimation of elements

Procedure

Analysis of minerals and trace elements of the tubers were done using Atomic Absorption Sepctrophotometer (AAS). Quantification of elements such as Calcium, Iron, Magnesium, Zinc were done. These crude extracts of hexane, chloroform, methanol and water were obtained for the reason that they would contain flavonoids, saponins, oils, fats, fibres and many other components. The tests later on with these crude samples proved to be positive through nutraceutical and qualitative photo chemical analysis.

3.9 ANTIMICROBIAL ACTIVITY

3.9.1 Collection Of Bacterial Cultures:

The Clinical Bacterial Strains were collected from Institute of Basic Medical Sciences, Chennai.

3.9.2 Micro-organisms tested: The Clinical Bacterial Strains were collected from Institute of Basic Medical Sciences, Chennai.The Bacterial strains studied are ,*Pseudomonas aeruginosa,Salmonella typhi,Staphylococcus aureus, Shigella flexenari , Esherichia coli, Bacillus subtilis*

• ***Staphylococcus aureus***: Gram +ve cocci cause boils, sinusitis and intoxication in human.

• ***Esherichia coli***: Gram –ve bacilli associated with infantile diarrhea, traveler's diarrhea, normally present in the gut.

• ***Pseudomonas aeruginosa***: It is an gram negative opportunistic pathogen causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections.

• ***Salmonella typhi***: a gram negative short rod causes typhoid fever.

• ***Shigella flexenari***: *gram negative bacteria,* causes bloody dysentery

• ***Bacillus subtilis***: a gram positive bacteria found in soil

3.9.3 Media Preparation

Three types of media are prepared in this assay

1. Nutrient agar

a. Nutrient agar ----------28 gm

b. Distilled water ----------1 L

Medium is dissolved and autoclaved at 121° C for 15 min and then cooled to 45° C. Addition of 40-50 ml media is made in sterile 14 cm diameter Petri plate and kept at room temperature.

2. Nutrient Broth

a. Nutrient broth----------0.8 gm

b. Distilled water ----------100 mL

Dissolved the broth and dispense approx. 3 ml nutrient broth in screw capped test tubes and autoclaved 121° C for 15 min.

3. Soft Agar

a. Agar Agar----------0.8 gm

b. Distilled water ----------100 mL

Dissolved the agar and dispense approx.7 ml soft agar in screw capped test tubes and autoclaved it at 121° C for 15 min and refrigerate it.

3.9.4 Test Sample preparation:

Crude extract: 3 mg/mL of DMSO

Procedure:

1. First day: inoculate single colony of bacterial culture in nutrient broth and incubate it at 37° C for 24 hrs.

2. Second day take soft agar tube, melt it and cool it up to 45° C then add 10 µl of fresh bacterial culture shake it well and then pour it on to the nutrient agar containing plate. Rotate the plate to make even

distribution of the culture, allow solidifying the lawn.

3. Make wells by using 6mm-diameter sterile borer and Mark the well with sample code.

5. Add 100uL of sample in respective agar well plate according to bacterial culture.

6. Other wells supplemented with DMSO and reference antibacterial drug serving as positive and negative control.

7. Incubate the plates at 37° C for 24 hrs.

8. Next day note down the result in terms of zone of inhibition in mm.

3.10 IN VITRO ANTIOXIDANT CAPACITY ASSAY

3.10.1. Total Antioxidant Capacity Assay

Principle

This assay is based on the principle of reduction of molybdenum (VI) to Molybdenum (V) by the extract and the subsequent formation of green phosphate molybdenum complex at acid pH.

Reagents

1. Reagent solution (0.6 sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate).
2. Ascrobic acid

Procedure

The working solutions (1-10mg/ml) of the samples were prepared by dissolving the extracts in the water. 0.2ml of the extracts were mixed with 2ml of the reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped with silver foil and incubated at 95c for 90 minutes. The tubes were then cooled to room temperature and the absorbance were measured at 695nm against a blank. Ascorbic acid was used as the standard. The total antioxidant capacity was expressed as acorbic acid equivalent (Raghavan Govindarajan *et al.,* 2003; umamaheswari *et al.,* 2008).

3.10.2. Determination of DPPH Radicle Scavening Activity

Principle

DPPH scavenging activity was measured by the slightly modified *spectrophotometric method.* 1,1-diphenyl-2-picryl-hydrazyl radical is scavenging by antioxidants through the donationof protons forming reduced DPPH. The electrons become paired off and the solution loses color depending on the number of electrons taken up. The color changes from purple to yellow after reduction, which is quantified by the decrease of absorbance at 517nm (Ajay Sharma *et al.,* 2007)

Reagents

1. Methanol
2. DPPH in methanol (0.004%)

Procedure

The banana extracts were dissolved in ethanol. A solution of DPPH in methanol (0.6mM) was prepared freshly. 3ml of this solution was mixed with 1ml of the samples of varying concentrations (1-10 mg/ml). The solution in the tube were vortexed and incubated in the dark for 30 min at room temperature. The decrease in absorbance was measured at 517nm. The control had equal volume of DPPH in methanol instead of extract. 5ml of methanol was blank. The percentage inhibition of the radicals due to the antioxidant property of the extract was calculated using the formula:

%inhibition = [(Acontrol-Asample)/Acontrol] 100

RESULTS & DISCUSSION

4. RESULTS AND DISCUSSION

4.1 Preparation of Seed Oil Extracts:

Different types of seasonal plant seeds based on their medicinal properties were collected from local markets of Tamil Nadu during January 2010.Healthy seeds(without weeds, other seeds, inert materials like insects, fungal bodies) were analysed.The samples are washed dried and set for grinding.The seeds are ground such that they are in between fine and coarse size.The appropriately ground seed samples are then used for extractions of oil by solvent extraction

Fig 4.1 Ladies Finger

Fig 4.2 Tomato

Fig. 4.4. Ground nut

Fig. 4.3 Drum Stick

4.2 Crushed seeds: The seeds were crushed between fine and coarse size.

Fig 4.5 Ladies finger

Fig. 4.6 Drumstick

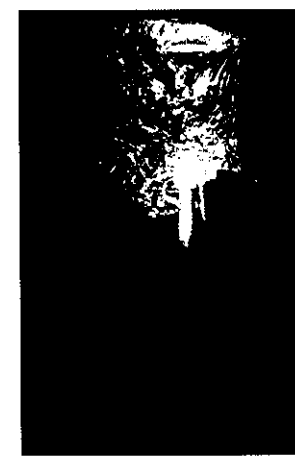

Fig 4.7 Tomato

4.3 Methanol Extracts

Fig4.8 Ladies Finger

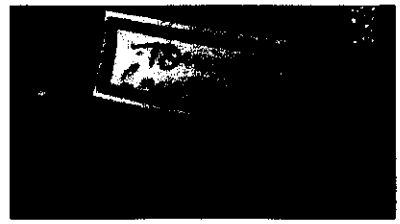

Fig4.9 Tomato

Fig4.10 Drum Stick

4.4 Acetone Extracts

Fig4.11 Drum stick

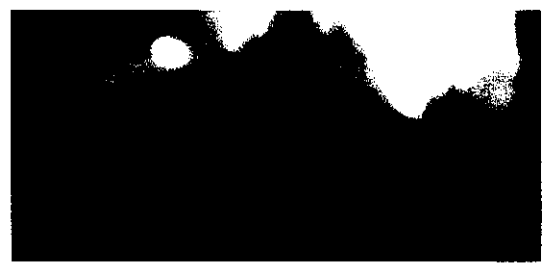

Fig4.12 Tomato

Fig 4.13 Ladies Finger

4.5. Proximate Analysis Results

Table 4.1- Proximate Analysis of the components present in the powdered seed samples.

S.No	COMPONENTS	RESULTS (ABELMOSHCHUS ESCULENTUS)	RESULTS (MORINGA OLEIFERA)	RESULTS (ARACHIS HYPOGAEA)	RESULTS (SOLANU LYCOPEI CUM)
1	Crude Fibre	5.0g	5.9g	8.7 g	4.2g
2	Crude protein	14.0g	13.2g	25.09 g	22.5g
3	Total Fat	6.58g	14.63g	47.58 g	29.6g
4	Total ash	3.84 %	4.14%	3.08%	2.0%
5	Carbohydrate	66.64g	63.2g	20.91 g	22.9g
6	Moisture	8.94%	4.83%	5.80%	5.23%
7	Energy	414.50Kcal	471.95Kcal	570 kcal	41 7kcal

The total protein value of Drum stick seed was found to be 13.2g and the total fat content neared to the value of 14.63g and the crude fibre of the plant sample reached to about 5.9g as in table 4.1. The ash content of the sample was found to be 4.14%and the calculated value of carbohydrate was found to be 63.2g.Moisture content was 4.83% and energy of 471.95Kcal.

The total protein value of Ladies finger seed was found to be 14g and the total fat content neared to the value of 6.58g and the crude fibre of the plant sample reached to about 5.8g as in table 4.1. The ash content of the sample was found to be 3.84%and the calculated value of carbohydrate was found to be 66.64g.Moisture content was 8.94% and energy of 414.5Kcal.

The total protein value of Ground nut seed was found to be 25.09g and the total fat content neared to the value of 47.58g and the crude fibre of the plant sample reached to about 8.7g as in table 4.1. The ash content of the sample was found to be 3.08%and the calculated value of carbohydrate was found to be 20.91g.Moisture content was 5.08% and energy of 570 Kcal.

4.6 Trace Elements

Since the result showed certain contents of sugar, ash and fibre, the research was further extended to find the presence of trace elements. It was found that the iron content was about . Hence ground nut is proved to be a major source of iron. Then found to contain Magnesium of about 2.09mg and Calcium content to about 62mg.Ladies Finger was found to have high Calcium content of 580mg, magnesium content of 220mg and iron content of 35mg.Drumstick seed was also found to have high calcium content of 280mg,magnesium content of 80mg and calcium content of 18mg

Table 4.2- Few Trace Elements present in the powdered seed samples

S.NO	TRACE ELEMENTS	(100 g dry weight) ABELMOSHCHUS ESCULENTUS	(100gdry weight) MORINGA OLEIFERA	(100 gdry weight) ARACHIS HYPOGAEA
1.	CALCIUM	580.0mg	280.0mg	62.0mg
2.	MAGNESIUM	220.0mg	80.0mg	2.09mg
3.	IRON	35.0mg	18.0mg	184mg

4.7 Antibacterial Activity Results

4.7.1 Agar Well Difussion Assay:

Presence of antibacterial agent was indicated by the growth inhibition of the bacterial strains and appearance of zone of inhibition. i.e. observe a clear zone where the growth of bacteria had not occurred. The cultures were kept at 4° C prior to testing. They were sub-cultured in liquid nutrient broth and incubate at 37° C for 18-24 hrs and then used for the screening

Table 4.3 – Activity of Bacteria using some Seed oil extracts

S.NO	BACTERIA USED	ACTIVITY (ABELMOSH-CHUS ESCULENTUS	ACTIVITY (MORINGA OLEIFERA)	ACTIVITY (SOLANUM LYCOPERSICUM)	ACTIVITY (ARACHIS HYPOGAEA)
1.	**Staphylococcus aureus**	11nm	11nm	-	-
2.	**Esherichia coli**	-	-	15mm	-
3.	**Pseudomonas**	-	-	-	-
4.	**Salmonella typhi**	-	-	19mm	-
5.	**Shigella flexenari**	-	-	-	-
6.	**Bacillus subtilis**	-	12mm	12mm	-

Acetone Extracts of Ladies Finger seeds and Drum Stick seeds showed non significant activity with *Staphylococcus aureus*. Acetone extracts of Tomato Seed showed good activity with *E.coli*. Extract of Tomato showed significant activity with Salmonella typhi . Exracts of Drum Stick and Tomato showed low activity with Bacillus subtillis.None of the other extracts showed activity with *Pseudomonas aeruginosa* and *Shigella flexenari*.

Antibacterial Assays

Fig4.14 *Salmonella typhi*

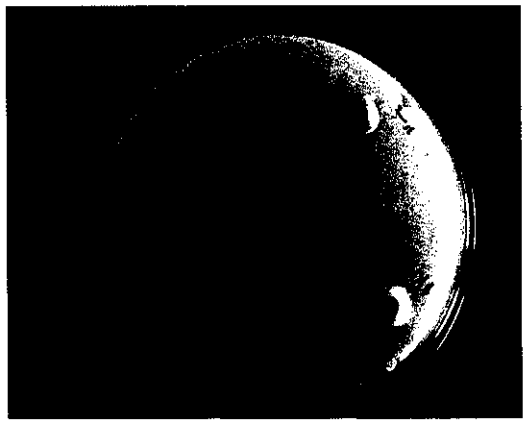

Fig4.15 *Escherichia coli*

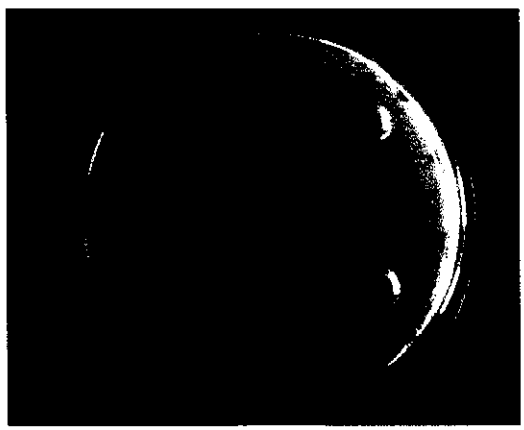

Fig4.16 *Bacillus subtilis*

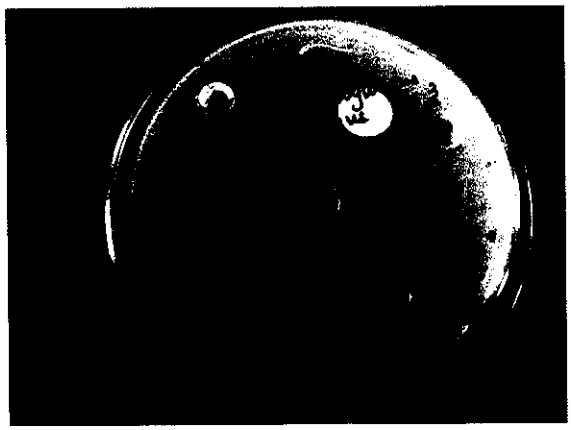

Fig4.17 *Staphylococcus aureus*

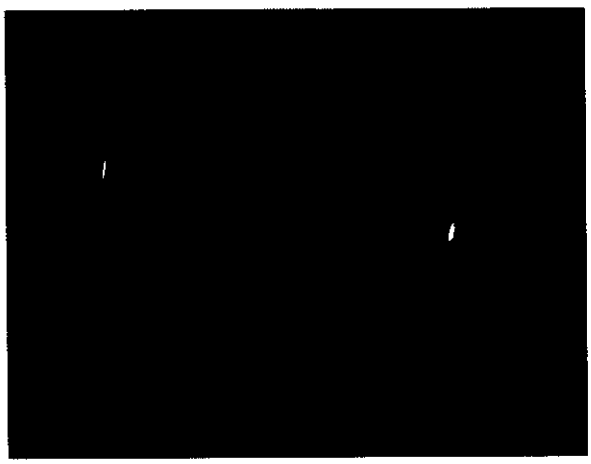

Fig4.18 *Shigella flexeneri*

Fig4.19 *Pseudomonas aeuroginos*

4.8Antioxidant Activity Results

4.8.1 Total Antioxidant Assay

Table 4. 4 Total Antioxidant Assay Values

S.No	Sample name	Ascorbic Acid Equivalent (µg/mol)
1.	*Abelmoshchus esculentus*	2.114
2.	*Solanum lycopersicum*	4.160
3.	*Moringa oleifera*	4.004
4.	*Arachis hypogaea*	2.012

From the table it is inferred that *Solanum lycopersicum* has highest total antioxidant in comparision to other variables.

4.8.2 DPPH radicle scavenging assay:

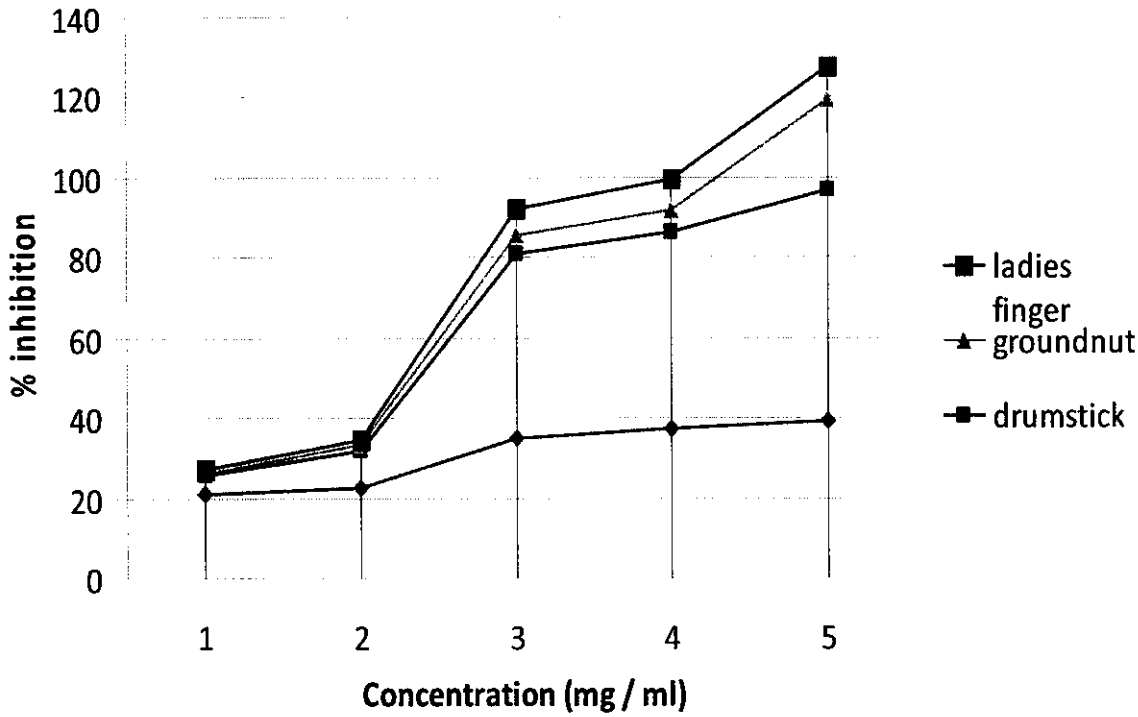

Fig 4.20 %Inhibition Vs Concentration graph

Perentage of DPPH radicles by the methanolic extract of Abelmoshchus esculentus,Solanum lycopersicum,,Moringa oleifera and Arachis hypogaea increases with increase in concentration.

CONCLUSION

5. CONCLUSION

Seed samples were collected from local market and powdered. A part of crushed seeds was used for solvent extraction. The nutritional and chemical compositions in the seed samples were analysed. The antioxidant as well as antimicrobial capabilities of solvent extracts of the seeds were studied. Antimicrobial activity of Tomato extract was found to positive in three bacterial organisms. Hence proving it is an effective antimicrobial agent if it would be used in larger cncentration. The four sample extracts showed to have significant antioxidant activity, with Tomato extract possessing highest antioxidant activity. The nutrional and chemical analysis significant showed presence of component indicating that the samples would be useful for formulation of dietary supplements and can be commercialized in order to meet increasing consumer demands.

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