A DISSERTATION ON

Analysis and Procurement of Pigeon Pea Through Residual, Contaminants and Physiochemical Parameters

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
FACULTY OF ENGINEERING & INFORMATION TECHNOLOGY
INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTER OF TECHNOLOGY
IN BIOTECHNOLOGY

BY
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M. Tech Biotechnology (IV Semester)
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UNDER THE SUPERVISION OF

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DECLARATION FORM

I, **Arooba Ilyas**, a student of **M. Tech Biotechnology (II Year / IV Semester),** Integral University have completed my six months dissertation work entitled **"Analysis and Procurement of Pigeon Pea Through Residual, Contaminants and Physiochemical Parameters"** successfully from **Regional Food Research & Analysis Centre, Lucknow** under the able guidance of **Dr. S.K. Chauhan (Director).**

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

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I wish her good luck and bright future.

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TO WHOM IT MAY CONCERN

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I wish her good luck and bright future.

Dr. Alvina Farooqui
Professor and Head
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Arooba Ilyas

ABBREVIATION USED

ACC : 1-aminol-cyclopropanoic acid

ASS : Atomic Absorption Spectrometry

Bt : Bacillus thuringiensis

DNA : Deoxyribonucleic Acid

DsPE : Decanting and Dispersive SPE

EPS : Exopolysaccharide

$FeSO_4$: Ferrous Sulfate

GCB : Graphitized Carbon Black

GC : Gas Chromatography

GoT : Grow-out-test

HM : Heavy Metals

IAA : Indole-3-acetic

HCL : Hydrogen Chloride

KOH : Potassium hydroxide

LC : Liquid Chromatography

M : Molar

Mbp : Mega base pairs

MQ : Milli Q

mL : Millilitre

MRLs : Maximum Residue Levels

PGO : Plant grow out tests

PCR : Polymerase Chain Reaction

Ph : Potential of Hydrogen

PSA : Primary Secondary Amine

RT : Room Temperature

SSR : Simple Sequence Repeats

UV : Ultraviolet

W/V : Weight per Volume

µl : Micro Liter

ng : Nano gram

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1.INTRODUCTION

A leguminous member of the Fabaceae family in the taxonomic order of, Fabales that can withstand moisture stress is the pigeon pea (*Cajanus cajan* (L.) Mill sp.). Other common names for the only species of the genus Cajanus grown commercially are red gramme, Gungo pea, no-eye pea, Gunga pea, and Congo pea (Akibode & Maredia, 2012; Njung' & Michael, 2013). Members of this genus were divided between Atylosia and Cajanus in older literature. Many taxa of Atylosia that were discovered to be linked to Cajanus were reclassified into Cajanus as a result of evidence from morphological, cytological, and chemo-taxonomical research. 32 species in this genus are found in Asia, Africa, and Australia (Pratap & Kumar, 2011; Smýkal et al., 2015).

The pigeon pea (*Cajanus cajan* (L.) Mill sp.), a major agricultural pulse crop in the Fabaceae family, with an estimated genome size of 858 Mbp and a diploid genome with 11 pairs of chromosomes (Dubey, 2011). According to the available information, the crop either originated in Africa or India. Vavilov (1951) came to the conclusion that India is the principal point of genesis of the domesticated pigeon pea due to the greater diversity of germplasm. This was further supported by De (1974) and Van der Maesen (1990), who claimed that the domesticated pigeon pea evolved in peninsular India, where its closest wild relatives (*Cajanus cajanifolia*) can be found in tropical deciduous woodlands, and that it was transported from this region to Africa around 2000 BC. Neolithic sites in Karnataka (Sanganakallu), its bordering states (Tuljapur Garhi in Maharashtra and Gopalpur in Orissa), as well as in Kerala in the south, have all yielded archaeological discoveries of pigeon pea that date to about 3400 years ago, i.e., approximately in the 14th century BC (Kassa et al., 2012; Songok et al., 2010).

With the importance of the crop in mind, efforts are being made to create high producing and disease resistant types. Even though hybrid pigeon pea development technology is about to be commercialised in India, types are still more common in pigeon pea growing regions. For these genetically pure cultivars to achieve their full potential, a reliable supply of high-quality seed is essential. Since pigeon peas are frequently cross-pollinated (3–45%), there is a chance that the seed could be contaminated. Before being released for commercial growth, the generated seed must undergo a field grow-out-test (GoT) for genetic purity, validating its authenticity and true-to-type characteristics. Because seed manufacturing might lead to some undesirable pollination occurrences, genetic purity testing is important in seed production. It can also be a key part of a successful quality assurance testing programme for seed manufacturers. The

quicker seed testing techniques will guarantee that seed lots match genetic purity requirements. Additionally, genetic purity testing aids in identifying potential variations, segregation, and seed mixtures in seed production batches. Genetic testing for variety verification makes ensuring that producers and consumers get the variety they anticipate. Pigeon pea field grow-out tests rely on morphological traits that will be assessed at various stages of crop growth. While the majority of the widely grown cultivars in southern India share similar physical traits, floral characteristics might vary. Additionally, the enhanced pigeon pea types grown in the southern region of India are medium to long durational, maturing in between 150 and 180 days. Therefore, the crop must be maintained until flowering, which is up to a minimum of 130 days in field GoT, in order to determine the authenticity of the variety. The seed dissemination for commercial cultivation will be delayed if the crop is kept for such a very long period without revealing the results of genetic purity, and the producer risk missing the advised sowing window. The final cost of the seed may increase as a result of storage and handling costs. Therefore, the development of quicker methods to evaluate genetic purity using physical, chemical, and molecular techniques is necessary. Faster outcomes also aid in "surety on purity" and the quicker availability of the stock for sales. Physical techniques include, in accordance with DUS criteria, morphological and seed characteristics such as anthocyanin pigmentation, leaf-plant-flower features, pod and seed colour, hilum colour, colour pattern, shape, length, width, L/B ratio, shine, type,1000 seed weight, etc. These features are used in plant grow out tests (PGO) or field grow out tests (GoT) to evaluate genetic purity. A requirement for seed certification in India is field GoT, an approved way to control the genetic integrity of a seed batch. For varietal identification in this method, a collection of qualitative and quantitative features known as descriptors relating to seed quality are now used. Some of the features, especially those that demonstrate quantitative inheritance, interact with the environment, making variety identification both verifiable and occasionally difficult due to the effect of G x E (genetic and environment) interaction that they disguise. Additionally, GOT is arduous, time-consuming, and extremely sensitive to labour and infrastructural abuse. It might take one entire growing season to complete. This technique is also highly seasonal and dependent on the soil's fertility for the emergence of unique features. Even though it is possible but expensive to do field GoT in controlled circumstances like a greenhouse or insect resistant nets. Seeds can be identified using chemical methods based on how they respond to tests like the KOH resistance test, $FeSO_4$ test, peroxidase test, phenol test, modified phenol test, etc. These tests are quicker than physical procedures, but depending on the test conditions, they may be very subjective. Sometimes different results can be obtained depending on the biochemical makeup of the

varieties being tested and off types. Some variations, nevertheless, might respond well to these techniques. No one chemical test was able to differentiate between all the variations. But to create the identity keys for each variant, separate chemical traits were utilised, and all the varieties were recognised using these identification keys. SSR markers are a quick way to screen for genetic purity in pigeon pea, and they can be used to tell one type apart from a group of others or from contaminants. For a group of types that are in the seed production cycle at a certain area, a distinct set of SSR markers can be developed. When compared to field GoT, these molecular approaches can be more expedient and affordable. These offer an objective way of identifying crop types and are also based on DNA sequence variation. These DNA- and PCR-based markers generate a significant amount of co-dominant polymorphism at the multiallelic level. These markers can be used with DNA of average quality, have great repeatability, and require little technical expertise. However, this technique requires a complex laboratory setup and a high level of automation (Nybom et al., 2014). Finding a quicker way to evaluate the genetic purity of the variety in question is a crucial step in the seed production cycle. The costs associated with each option must also be assessed.

Pest control agents are compounds known as pesticides. Insecticide, nematicide, molluscicide, piscicide, avicide, rodenticide, bactericide, insecticide, animalicide, microbicide, fungicide, and lampricide are some examples of these. Herbicides are the most widely used of them, making for around 50% of all pesticide use worldwide. The majority of pesticides are designed to act as crop protection agents, or plant protection products, which often shield plants from weeds, fungi, or insects. Target organism (e.g., herbicides, insecticides, fungicides, rodenticides, and pediculicides - see table), chemical structure (e.g., organic, inorganic, synthetic, or biological (biopesticide), and physical state (e.g., gaseous (fumigant)) can all be used to categorise pesticides. Microbial and biochemical pesticides are both types of biopesticides. Recent years have seen the development of insecticides made from plants, or "botanicals". These include scilliroside, strychnine, rotenoids, pyrethroids, and nicotinoids (Ngegba et al., 2022). Chemical insecticides are used to manage significant yield losses in the pigeon pea crop. Growers heavily rely on chemical pesticides due of the apparent knockdown effect of pesticides, more so by insecticides on the pod borer, pod fly, and pod bug complex. The application of pesticides to control different types of pests may result in residues exceeding their MRLs and have toxicological implications for the environment. The maximum residue levels (MRLs) for pesticides used in pigeon pea are established between 0.01 and 1.50 mg kg^{-1}.

Farmers can save money by using pesticides to stop crop losses to insects and other pests. A prohibition on pesticides would cause food prices to increase, people to lose their employment, and there might be more hunger in the globe. On the other hand, those exposed to pesticides may have immediate and delayed health impacts. Pesticide exposure can have a range of harmful health impacts, from minor skin and eye irritation to more serious ones that disrupt the nervous system, hearing, mimic hormones and lead to reproductive issues as well as cancer (Sabarwal et al., 2018). The intensity of pesticides' harmful effects on people and other non-target species varies on how often and how much they are exposed to them. The rate of compound absorption, distribution within the body, metabolism, and removal from the body all affect toxicity. Commonly used pesticides like organophosphates and carbamates work by preventing the function of acetylcholinesterase, which stops acetylcholine from being broken down at the brain synapse. Symptoms of too much acetylcholine include nausea, tremors or cramping in the muscles, confusion, dizziness, and nausea.

The usage of pesticides causes a number of environmental issues. Pesticide drift is when airborne pesticide particles are transported to neighbouring locations by the wind and may end up polluting them. Pesticides are one of the main contributors to water pollution, and some of them are persistent organic pollutants that contaminate soil and flower nectar and pollen as well. Organisms frequently retain chlorinated hydrocarbon insecticides virtually indefinitely because they dissolve in lipids and are not expelled. These chlorinated hydrocarbons (pesticides) become more concentrated at each step of the food chain through a process known as biological magnification. Pesticides should be able to degrade or at the very least swiftly deactivate in the environment to minimise any unwanted effects (Karimi et al., 2021; Parra-Arroyo et al., 2022; Raffa & Chiampo, 2021). Both the innate chemical features of the chemicals and environmental processes or situations are to blame for this decline in pesticide action or toxicity. For instance, in an aerobic environment, the presence of halogens inside a chemical structure frequently slows down decomposition. Although adsorption to soil may slow the flow of pesticides, it may also lessen their bioavailability to microbial degraders.

Objectives

The objectives of the present work are:

1. Estimation of physio-chemical parameters and analytical parameters of pigeon pea (Cajanus cajan)
2. Analysis of pesticide residue using LC-MS/MS in pigeon pea.

2.REVIEW OF LITERATURE

The global demand for protein is significantly influenced by plant protein. It meets around 65% of the protein needs of people worldwide. Pulses are a general term for dried seeds of edible legumes. Only around 20 of the 13,000 species of legumes are regularly ingested by people. Popular names for pulses or legumes include "poor man's meat" and "rich men vegetable". Legumes are the second-best source of proteins, carbs, including fibre, certain minerals, and B-complex vitamins after cereals. They are two to three times as protein-rich as cereal grains in terms of nutrition. Pulses can also be grown in drought-prone locations, utilised as fuel, feed, and soil conditioners, and they are highly water-efficient. They also help to increase soil fertility by fixing atmospheric nitrogen in the soil.

Figure 2.1: Pigeon pea plant showing pods

The Phaseoleae family includes the pigeon pea (*Cajanus cajan* L.), a diploid legume crop species (2n = 2x = 22). There are 32 species in the genus Cajanus, but only *Cajanus cajan* is grown as a crop. Pigeon pea seeds typically include 85% cotyledons, 14% seed coat, and 1% or less embryo inside their seed and are rich in a variety of dietary components. The cotyledons are abundant in carbohydrates (66.7%), whereas the embryo contains the majority (about 50%) of the seed protein. The proteins found in legume seeds can be broadly divided into two groups:

storage proteins, which are created during seed development, and metabolic proteins, which are involved in regular cellular processes. Pigeon peas have a protein level that ranges from 15.5% to 28.8% and is influenced by both genetic and environmental influences (K. B. Saxena et al., 2023). Similar to other legumes, pigeon pea protein has an excess of lysine, which is a limiting amino acid in cereals, and a deficiency in sulfur-containing amino acids (methionine and cysteine) (Acharjee & Sarmah, 2013). The seed coat contains fibre to an extent of around one third. Pea seeds are a rich source of new medications that are used as pharmaceutical intermediates, lead compounds in synthetic drugs, and components in modern and traditional treatments. The antihelminthic, antiparasitic, antibacterial, antifungal, anticancer, antioxidant, and antidiabetic properties of the leaves and seeds are well documented. To encourage breastfeeding, the leaf and seed are put to the breast as a poultice. Legumes with high trypsin inhibitor activity impede protein metabolism, while phytate phosphorus hinders the absorption of minerals (Samtiya et al., 2020). Despite being widely grown, the NE region of India's pigeon pea farming is not very promising. This crop is being grown sporadically in this area. The crop's low production could be the main cause. The lack of improved cultivars, inadequate crop husbandry techniques, and exposure to a variety of biotic and abiotic challenges in the environment may all be to blame for the comparatively low crop yields. Therefore, it is urgently necessary to create pigeon pea types with large yields. So far, no systematic attempts have been made to compile data on the nutritional makeup of the pigeon pea germplasm collection.

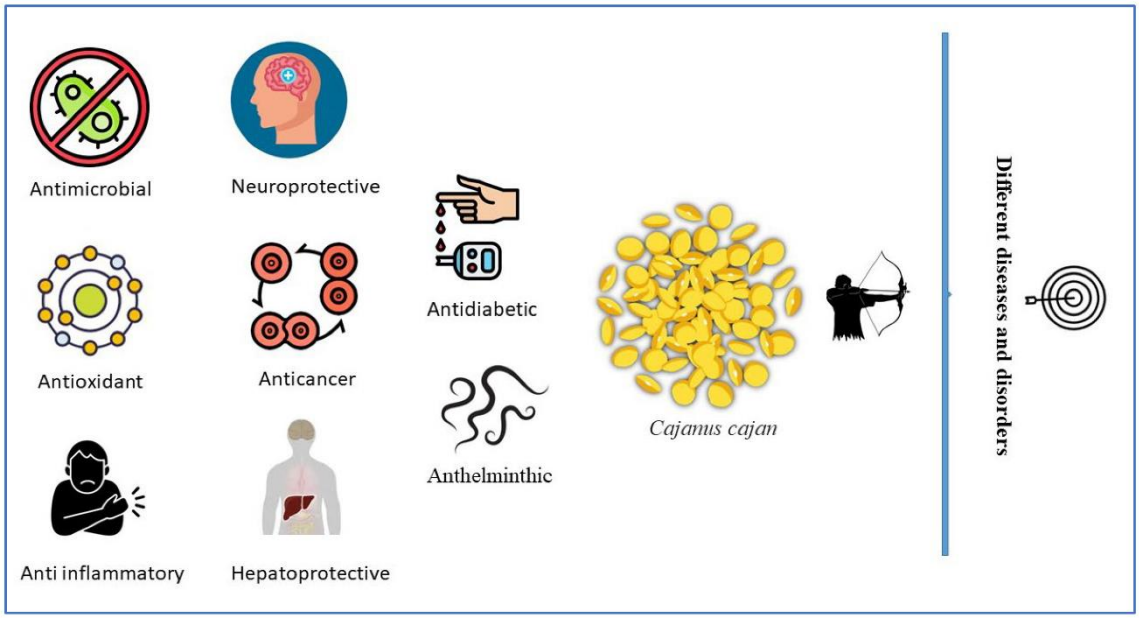

Figure 2.2: Uses of pigeon pea.

Pigeon pea, one of the edible legumes, is essential for human nutrition and for meeting socioeconomic requirements for revenue per unit area in rainfed or dryland farming. Since pigeon pea agriculture depends not only on the creation of improved varieties but also on an adequate supply of seeds that are true to type and confirm the desired characteristics as stated by the breeder. Testing for genetic purity is essential in giving seeds with certainty on purity in order to assure the authenticity of seed lots. Due to the long duration of the pigeon pea and the nearly identical morphological characteristics of all produced varieties, with the exception of a few floral traits, it is crucial to create quicker genetic purity testing technologies that can discriminate between contaminants and varieties. Faster processes guarantee the timely delivery of high-quality seed to farmers while reducing the pressure on storage. A variety's commercialization and greater adaptability also depend on higher genetic purity.

The family of flowering plants known as Leguminosae contains 18,000–19,000 species and 650–750 genera. The four subfamilies of this family—Caesalpinioideae, Mimosoideae, Papilionoideae, and Swartzioideade—are collectively referred to as legumes. Legumes are high in protein (20–40%), starch (50–60%), fat (2–3%), dietary fibre (0.7–6.2%), and vitamins and minerals (0.7–6.2%). Legumes can be an excellent source of functional foods because they also have a glycemic index that is low in value and bioactive substances with antioxidant capabilities. Legumes may have a good impact on health, serving as a supplement for those with diabetes mellitus and reducing the risk of heart disease, obesity, and bone disorders (Craig, 2010; Rebello et al., 2014). To improve the nutrient profile and functional impact of the end product, legumes are frequently utilised as a cereal substitute. Legumes can therefore be an alternative to meet nutritional demands and combat a number of ailments, particularly in developing nations. A growing demand for protein-rich food sources exists in industrialised nations due to factors such as population growth, insufficiently fertile soil, a diet high in cereal, and high food prices. Legumes have been the main food supply in many nations, including the Middle East, North Africa, and South Asia. Examples include pigeon pea, chickpea, and lentils in South Asia, kidney beans in Latin America, and chickpea, lentils, and faba beans in North Africa. Soybean, red kidney bean, and mung bean are still the only types of legumes that are commonly consumed in Indonesia. On the other hand, Indonesians have not made the best use of a variety of legumes, including pigeon pea.

Cajanus cajan (L.) Mill sp., sometimes known as pigeon pea, belongs to the Fabaceae family. Pigeon peas are an annual crop that may be produced in tropical and subtropical regions because they are more tolerant of drought and high temperatures than other crops (Sultana et

al., 2014; Upadhyaya et al., 2012). The plant has a taproot that is 2 metres deep and ranges in height from 1-4 metres. Pigeon pea pods have 2 to 9 hairy or striped seeds/pods and are flat, dark purple or green. Seed weight varies from 4 to 25 g per 100 seeds. There are several different names for pigeon pea in some languages, including guand (Portuguese), tur and arhar (Hindi), gandul (Spanish), ervilba de Congo (Angola), poid d'Angole and poid de Congo (French), red gramme, and congo bean (English).

Pigeon peas are typically eaten as a vegetable, yet a few culinary items still use pigeon peas. Therefore, study on the physical traits, nutrients, and anti-nutrients composition of pigeon pea is required to expand the diversification of food products based on pigeon pea. Processing, storing, and developing equipment for processing require knowledge of the physical properties of legumes. Legumes' ability to retain moisture and their seed weight are related to how they are cooked. Less time is needed to cook legumes with higher hydration capacities, which affects consumer liking for the seeds. When developing machinery for sizing and grading, consideration must be given to the size and shape of legume seeds. The formulation and use of food-based dietary guidelines, public health nutrition, and the assessment of dietary quality all benefit from knowledge of the composition of the nutrients.

2.1. Contamination in Pigeon Pea

The two main pollutants in pigeon pea are Lathyrus sativus (kesari dal) and metanil yellow. Azo dyes are a subset of the dye family that contains Metanil Yellow (Acid Yellow 36). It is employed as a pH indicator in analytical chemistry and changes from red to yellow between pH 1.2 and 3.2. Because of its eye-catching yellow tint, particularly in India, Metanil Yellow has been added to turmeric powder and Arhar dal despite being an illegal food colour (Gupta, 1987). Animal studies show that metanil yellow is neurotoxic and poisonous to the liver.

Microbiological tests showed that pigeon pea seeds can be heavily contaminated with fungi, like Aspergillus (71.42%) and Fusarium (26.19%) being the most common. *Aspergillus ochraceus*, *Aspergillus parasiticus*, *Aspergillus flavus*, and *Fusarium oxysporum* are among the prominent fungal contaminants (Lomate & Hivrale, 2011). These result in poisonous secondary metabolites that could be very dangerous to our health (Pandey et al., 2013).

Plant development and productivity are hampered by heavy metal toxicity, which also poses serious health risks to people. Many metals and metalloids, including arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), zinc (Zn), cobalt (Co), aluminium (Al), and

chromium (Cr), cause extreme toxicity when they reach the soil agro-ecosystem through either natural or manmade mechanisms (Niyoifasha et al., 2023). While anthropogenic sources include significant mining, metal smelting, the use of chemical fertilisers, industrial/sewage discharge, and coal combustion, natural sources of heavy metals (HM) contamination include weathering of rocks, soil erosion, forest fires, and volcanic eruptions. The typical geochemical cycle of metals and elements has been disrupted by rapid industrialization and technological breakthroughs, which has increased the accumulation of these materials in soil horizons (Rajkumar et al., 2013). It is presently believed that increased bioaccumulation of heavy metals above the threshold level poses an immediate threat to the ecosystem and environment due to its detrimental effects on the natural food chain and microbial flora. In plants, heavy metals are taken up by the roots and transported to the shoots, where they significantly harm internal organelles like the mitochondria and chloroplast. This reduces energy production and places oxidative stress on the plant, which in turn affects plant morphology and survival rates (Mahapatra et al., 2020). Some kinds of plants are tolerant to heavy metals or even hyper-accumulators, yet many plants are susceptible to them. A subset of bioremediation known as phytoremediation employs plants to remove contaminants from contaminated soils. It works well on contaminated settings where the toxins are dispersed throughout the plant roots. Through a number of processes, the rhizosphere bacteria that live in the plant's root zone contribute significantly to the phytoremediation process. The majority of the rhizosphere's physical and chemical processes have an immediate effect on the root system. It is generally known that the interaction between plants and microbes affects how effectively metals are extracted. The rate of heavy metal accumulation in plants is accelerated by a number of mechanisms, including the production of exopolysaccharide (EPS), rhizosphere acidification through organic acids, siderophores, indole-3-acetic (IAA), or 1-amino1-cyclopropanoic acid (ACC) deaminase, as well as the release of growth-restricting nutrients from the soil (Caracciolo et al., 2021).

2.2. Pesticides

Pesticides are substances that are frequently employed in agriculture, forestry, parks, industrial areas, sports venues, and other settings. They are used for maintaining lawns, controlling vegetation in industrial settings, and promoting public health (by eradicating rodents and insects that can spread disease). They repel, prevent, mitigate, or eliminate weeds and/or pests as part of their mode of operation. Insecticides, herbicides, bactericides, fungicides, miticides, molluscicides, nematicides, wood preservatives, and rodenticides are some of the several types

of pesticides that can be categorised based on their intended use. Pesticides can be divided into organochlorines, organophosphorus, carbamates, chlorophenols, and synthetic pyrethroids based on their chemical makeup. Direct releases of pollutants into the environment occur as a result of mishaps such transportation spills, percolation from disposal sites, storage, or industrial operations. When pesticides are applied to crops, rain, irrigation water, and wind can carry them to the soil. They then migrate from the soil into surface watercourses and groundwater through infiltration and runoff processes, as well as through wastewater treatment facilities. In addition to the aforementioned, a number of pesticides are pervasive substances that linger in soil and sediments due to their low bioavailability. Pesticides are therefore extremely hazardous and can lead to chronic human problems while damaging the ecosystem and biodiversity (Ali et al., 2021).

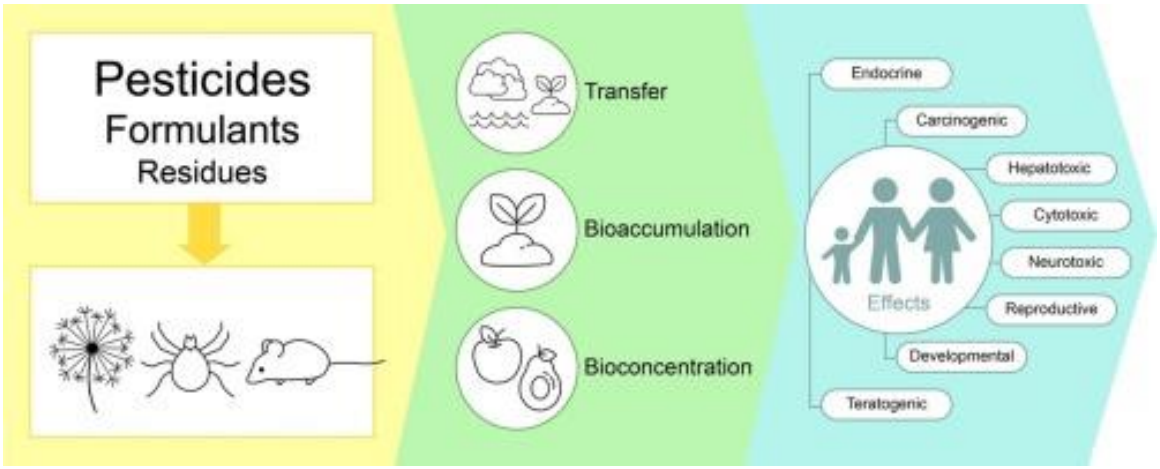

Figure 2.3: Impact of pesticides on human health.

Chemical pesticides have a number of negative effects on environmental and human health. Chemically created pesticide residues can be found in soil, water, food, and other places. These residues can cause phytotoxicity, physiological disease, mortality, and population shifts in a variety of organisms (Bourguet & Guillemaud, 2016). They can also result in genetic diseases. Along with biomagnifications and bioaccumulation, they can have serious implications once they reach food systems (Singh et al., 2017). Utilising biopesticides is a promising alternative. The formulations known as "biopesticides" are made from naturally occurring compounds that work to decrease insect populations through non-toxic, ecologically acceptable means. Biopesticides can be made from organisms, their products, or their byproducts that can be used to manage pests. These sources can be microbial, plant, or animal. The Central Insecticide

Commission and Registration Commission currently has 970 biopesticide products registered. It is the top government agency in India for the use of all biopesticides. Of the overall biopesticide output, bacterial, fungal, viral, and other (plant, pheromone) biopesticides accounted for 29,664 and 1%, respectively (Ekström & Ekbom, 2011). With roughly $3 billion and 5% of the global crop protection industry, biopesticides have a limited part of the crop protection business. The biopesticides made from neem, *Bacillus thuringiensis* (Bt), nuclear polyhedrosis virus, and *Trichoderma* are the most often produced and utilised in India. 90% of all microbial biopesticides now on the market are generated from the entomopathogen Bt. Neem-derived herbal biopesticides are safe for the environment and very effective against a variety of insects, nematodes, and fungi. Azadirachtin, a natural antifeedant, insect growth regulator, and fungicide, is found in neem tree (*Azadirachta indica*) seeds (Boudh & Singh, 2018). Through farm runoff and soil erosion, agricultural pesticides can contaminate nearby water bodies, resulting in poor water quality and the possible presence of aquatic creatures. Additionally, pesticides can cause losses in fisheries by increasing mortality, obliterating necessary insects and other invertebrates, and killing young, vulnerable fish. Fish deaths were caused by chlorpyrifos, an organophosphate insecticide that is very hazardous to fish, in rivers close to fields that had been treated (Kumar et al., 2021).

3.MATERIALS AND METHODS

3.1. Material

Pigeon pea

Pigeon pea, was collected from local market. It is locally known as ‘daal’. The scientific name of pigeon pea is Cajanus Cajan.

3.2. General Glassware and Apparatus

- Beakers (different sizes)
- Conical flasks with and without lids (different sizes)
- Round bottom flasks (different sizes)
- Standard volumetric flasks (different sizes)
- Pipettes (different sizes)
- Burettes (different sizes)
- Measuring cylinders (different sizes)
- Air condensers
- Buchner funnels (different sizes)
- Water condensers
- Distillation heads
- Wash bottles (different sizes)
- Separating funnels (different sizes)
- Petri dishes (different sizes)
- Weighing balances (up to milligram)
- Weighing balances (up to gram)
- Falcon tubes (different sizes)
- Whatman filter papers (different numbers)

3.3 CHEMICAL ANALYSIS

3.3.1. To determine the moisture content in pigeon pea.

Requirements: Sample, Patri dish, Oven, weighing balance.

Procedure: 5g of sample was taken in a clean and dry petri dish and placed in an oven for 3-4h at 130°C. After 3-4h, petri dish was taken out of the oven, and cooled in the desiccator, and weighed.

Formula used

Moisture % = W1-W2*100/W1-W

where

W1 = weight in gm of the petri dish with the material before drying.

W2= weight in gm of the petri dish with the material after drying.

W= weight in gm of the empty dish.

Calculation

Moisture (%) = 73.0168-72.5253 x100/73.0168-67.9938

Moisture (%) =9.84

3.3.2. To determine the ash content in pigeon pea

Requirements: Sample, silica crucible, muffle furnace, weighing balance.

Procedure: 5g of sample was taken in a silica crucible and kept in a muffle furnace at temperature range 500-600°C for 24 h. The crucible was taken out of the furnace and kept in a desiccator to cool. The weight of the crucible was taken number of times till it was constant.

Formula used

Total ash on dry basis (% by weight) = (W2-W)x100/W1-W

Where

W2 = weight in gm of the crucible.

W = weight in gm of empty crucible.

W1 = weight in gm of the crucible with the dried material taken for test.

Calculation

Total ash on dry basis (% by weight) = (19.7322-19.5500)*100/24.5828-19.5500

Total ash on dry basis (% by weight) = 3.62

3.3.3. To determine the fat content in pigeon pea.

Requirements: Sample, hydrochloric acid, ethanol, water bath.

Procedure: 2.0 g of sample was taken in a conical flask (100mL) containing 2 mL of ethanol and 10 mL of 8N hydrochloric acid. The conical flask was covered with a silver foil and heated on a water bath until the silver foil cracked. After fat digestion, the content was filtered using a Whatman filter paper (41/1 N). Filter paper was washed using hot water till it reaches to the pH 7. The filter paper was kept in hot oven for 2h. After drying, filter paper was set in Soxhlet assembly for washing with the petroleum ether. After washing the flask was kept in a oven for 4-5h. Flask was taken out of the oven and cooled at room temperature in desiccator and weighed to get dry material.

Formula used

Total fat % = M2-M1 x100/M

Where

M1 = weight of empty conical flask

M2 = dry weight conical flask along with the fat

M = weight of the sample

Calculation

Total fat (%) = 157.1906-157.0598 x100/2.0321

Total fat (%) = 6.43

3.3.4. To determine acid insoluble ash in pigeon pea.

Requirements: dilute hydrochloric acid (approx. 5.5 N)

Procedure: To the ash obtained from the previous experiment in a 100 mL conical flask then add 25mL hydrochloric acid in it and heated on a hot plate to boil. The reaction mixture was filtered with Whatman filter paper. The residue was with distilled water to remove acid. The filter paper was kept in a crucible and put in oven maintained at 130°C for 2h. The crucible along with filter paper was placed in muffle furnace for 6h maintained between 500-600°C. The crucible was taken out of the furnace and cooled in a desiccator and weighed.

Formula used

Ash insoluble in dil. HCl on dry weight basis = (W2-W)x100/W1-W

Where

W2 = Weight in g of crucible with the acid insoluble ash.

W= weight in g of empty crucible

WI= weight in g of the crucible with the dried material

Calculation

Ash insoluble in dil. HCl on dry weight basis = (24.5828-19,5500) x100/19.5614-19.5500

Ash insoluble in dil. HCI on dry weight basis = 0.22 %

3.3.5. To determine ca in pigeon pea.

Requirements: Hydrochloric acid (1N), nitric acid (15N), ammonium hydroxide, ammonium oxalate, potassium permanganate, Whatman filter paper (40).

Procedure: Ash which was obtained from 5g of sample was taken in a 100 mL conical flask containing 5 mL hydrochloric acid and 0.5mL nitric acid and heated on a hot plate till it boiled. Reaction mixture was filtered using Whatman filter paper in a 100mL volumetric flask and volume was made up to 100mL using distilled water. 50 mL solution was taken out from the volumetric flask in a conical flask (250mL) to the same conical flask 50 mL of distilled water was added. 2-3 drops of methyl orange solution were also added to the same conical flask to get pink colour. Ammonium hydroxide was added to set the pH of the solution 5.6 and the color of the solution changed to yellow. Hydrochloric acid was again added to the solution to get pink color. 50 mL distilled water was also added to the solution and heated to boil. 10 ml ammonium oxalate solution was added to the solution. The solution was filtered through Whatman filter paper and paper was washed with hot water till pink colour disappeared

followed by washing with ammonium hydroxide (to make the volume 100mL) and 1.5N sulfuric acid further to make volume up to 130 ml. Filtrate was heated on a hot plate at 70-800c fir 4-5 min. the residue adhered on filter paper was also dissolved in the same solution and titrated against 0.05N potassium permanganate. Pink colour showed the end point.

Formula used

Calcium = T.V.X10x1000/Sample weight of ash x50

Calculation

Calcium = 3.3 x10x1000/5.0286 x50

Calcium =131.24mg/100g

3.3.6. To determine alcoholic acidity in pigeon pea.

Requirements: Neutral ethanol, sodium hydroxide solution (0.05 N), phenolphthalein indicator, stoppered conical flask (250mL), Whatman filter paper (1).

Procedure: 5 g of sample was taken in a 250mL stoppered conical flask containing 50mL neutral ethanol and swilled gently and left for 24h with occasional swirling. The solution was filtered through a dry filter paper. 10 ml of the alcoholic extract was titrated against sodium hydroxide solution using phenolphthalein indicator till a pink colour was obtained.

Formula used

Alcoholic acidity with 90% alcohol calculated H_2SO_4 on dry basis =

Titre value x 24.32 x normality of NaOH/weight of sample (dry weight)

Calculation

Alcoholic acidity with 90% alcohol calculated H,SO, on dry basis = 0.5x24.52x0.05/5.0090 = 0.122

3.3.7. To determine the fibre in pigeon pen.

Requirements: Dil. Sulphuric acid (1.25%), sodium hydroxide solution (1.25%), ethanol (95%), petroleum ether.

Procedure: 2.5 g of sample was weigh and was transferred to the dried crucibles.

The crucible was placed into rubber adopters of fibro Tron extraction unit and ensure proper sealing of crucible against the adaptor rubber. After that acid wash and alkali wash done and instrument was switched on and temperature was maintained 350 Celsius. Drained acid and alkali sample was washed with distilled water.

Crucible was weighed and reading was recorded.

Formula used

Fibre (5% by wt.) = (W1-W2) x 100/W

W1 = weight in g of Gooch crucible and contents before ashing

W2 weight in g off Gooch crucible containing asbestos and ash

W= weight in g of the dried material taken for the test

Calculation

Fibre (5% by wt.) = (42.2402 -42.2279) x100/0.5010

Fibre = 2.45%

3.3.8. To determine protein in pigeon pea using Kjeldahl instrument

Requirements: Kjeldahl digestion flask 500 or 800mL, Kjeldahl distillation apparatus, conical flask 250ml, Burette 50ml, conc. sulphuric acid, sodium hydroxide solution (0.1N), methyl red indicator, sulphuric caid solution (0.IN), 45% sodium hydroxide solution, hydrochloric acid 0.1N.

Procedure: 0.5 g sample, 0.5 g cupric sulphate and 3g potassium sulphate were taken in digestion tube and to it 10 ml conc. Sulphuric acid was added. The aliquot of digested sample was distilled with 45% sodium hydroxide solution in Kjeldahl distillation set. The ammonia was distilled out from the digested sample and collected in 4% boric acid solution containing a drop of methyl red indicator. After collecting condensed nitrogen in a conical flask it was filtered with 0.1N hydrochloric acid to pink end point.

Formula used

Protein (%) = 14.1 x 6.25 x T.V. x 0.1016 x100/ sample wt. x1000

Calculation

Protein (%) = 14.1 x 6.25 x 12.3 x 0.1016 x100/0.5049 x1000

Protein (%) =21.67

3.3.9. To determine carbohydrate in pigeon pea.

Carbohydrate content was calculated by subtracting the moisture, protein, fat and ash content from the total mass.

Calculation

Carbohydrate(%) = [100 - (moisture + protein + fat + ash)

Carbohydrate =[100-(9.84+21.67+6.43+3.62] = 58.44

3.3.10. To determine energy in pigeon pea.

Calculation

Energy (Kcal) = 4(carbohydrate + protein) +9x fat

Energy = 4(58 + 21.67) + 9 x 6.43

Energy =378.31 kcals

3.4. Spectroscopic Analysis

3.4.1. Determination of Trace Metals

Flame (AAS)

Figure 3.1: Flame atomic absorption spectroscopy

Flame atomic absorption methods are referred to as direct aspiration determinations. They are normally completed as single element analyses and are relatively free of inter element. For some elements, the temperature or type of flame used is critical. If flame and analytical conditions are not properly used, chemical and ionization interferences can occur. Different flames can be achieved using different mixtures of gases, depending on the desired temperature and burning velocity. Some elements can only be converted to atoms at high temperatures. It involves measuring the sample of interest in a series of samples of known concentration and all prepared under the same conditions.

Mineral elements compositions were estimated in local pigeon pea sample by standard analytical methods coupled to the atomic absorption technique. The estimated significant quantities of three essential elements such as Iron (Fe), Zinc (Zn) and manganese (Mn) were determined by the atomic absorption spectrophotometer.

SAMPLE PREPARATION

Method A (microwave acid digestion)-

0.5 g of the dried (105 C) sample was digested with 6 cm3 of concentrated HNO3 and 2 cm3 of concentrated HCI in closed polytetrafluoroethylene (PTFE) vessels in a microwave oven. A three-stage protocol (as below) was used. After digestion the solution with a solid phase was placed into the 100 cm3 volumetric flask, filled to the mark with Type I (ISO 3696) deionized water of resistivity > 10 MΩ cm and filtered through a filter paper (pore size 8 um, medium porosity) to a PE bottle.

UV-visible spectrophotometer

Figure 3.2: UV-visible spectrophotometer

Ultraviolet-visible (UV-Vis) spectrophotometry is a technique used to measure light absorbance across the ultraviolet and visible ranges of the electromagnetic spectrum. When incident light strikes matter it can either be absorbed, reflected, or transmitted. The absorbance of radiation in the UV-Vis range causes atomic excitation, which refers to the transition of molecules from a low-energy ground state to an excited state. Before an atom can change excitation states, it must absorb sufficient levels of radiation for electrons to move into higher molecular orbits.

3.4.2 Determination of Total Phenolic Contents

Phenols, the aromatic compounds with hydroxyl groups, are widespread in the plant kingdom. They occur in all parts of the plants. Phenols are said to offer resistance to diseases and pests in plants. Grains containing a high amount of polyphenols are resistant to bird attack. Phenols include an array of compounds like tannins, flavanols, etc. Total phenol estimation can be carried out with the Folin-Ciocalteu reagent.

Requirements: 80% Ethanol, Folin-Ciocalteu Reagent, Na2CO, (20%) Standard (100 mg Catechol in 100mL Water), Dilute 10 times for a working standard

Procedure: Weigh exactly 0.5-1.0 g of the sample and grind it with a pestle and mortar in the 10-time volume of 80% ethanol. Centrifuge the homogenate at 10,000 rpm for 20 min. Save the supernatant, Re-extract the residue with five times the volume of 80% ethanol, centrifuge, and pool the supernatants. Evaporate the supernatant to dryness. Dissolve the residue in a known volume of distilled water (5 mL). Pipette out different aliquots (0.2-2 mL) into test tubes. Make up the volume in each tube to3 mL with water. Add 0.5 mL of Folin-Ciocalteu reagent. After 3 min, add 2 mL of 20% NaC03, and solution to each tube. Mix thoroughly. Place the tubes in boiling water for exactly one min, cool, and measure the absorbance at 650 nm against a reagent blank. Prepare a standard curve using different concentrations of catechol.

Calculation

From the standard curve find out the concentration of phenols in the test sample and express it as mg phenols/100g material.

3.4.3. Determination of Total Flavonoid Contents

The total flavonoid content was determined with colorimetry method of aluminium chloride with a spectrophotometer as the absorbance measurements and quercetin as the standard. A sample of 2 5mg of ethanol extract of oil sample was dissolved in 25 ml of methanol, and then it was diluted until the concentration of the solution was 300 ppm, After 2ml of sample with a concentration of 300ppm was obtained, 0.1 ml of AICI3, 0.1 ml of sodium nitrate and 3ml of distilled water were added to the solution. The absorbance was determined using a visible spectrophotometer at a wavelength of 510nm. The flavonoid content was expressed in mg equivalent quercetin/g samples (mg Q/g) and calculated based on the equation:

TOTAL FLAVONOID CONTENT=X. v. /W

Where

X = concentration (ppm)

V= volume of sample solution (extract) (ml)

W= sample weight (g)

3.5. QuEChERS Multi-Residue Method:

Step 1: Homogenization and Sampling

- The food sample is homogenized to increase the surface area available for extraction and to ensure a representative sample.
- The homogenizing process generates heat that can drive off the pesticides of interest and compromise the analysis. To minimize this, the sample is typically homogenized in a cryogenic/sub ambient condition.
- Typically, 5 grams of the homogenized sample is transferred to a 50 mL centrifuge tube. For dry samples, especially those containing a water content <25% (e.g., flour, dried fruits, spices), the sample size may need to be reduced and water added prior to homogenization.

Step 2: Addition of Extraction Solvent

- An extraction solvent is added to the centrifuge tube. It is recommended to use Acetonitrile because it can be easily separated from water. Because most food samples typically contain 80-95% water, separation of the sample from water is very important to this application. In general, 10-15 mL of extraction solvent is added to the centrifuge tube.
- An Internal Standard solution can be added at this step to monitor extraction efficiencies and aid in the quantitation of the target analytes.

Step 3: Liquid Extraction

- Once the solvent has been added to the tube, the tube is shaken vigorously for 1 minute.

Step 4: Buffering and Drying

The three accredited methods differ slightly on the amounts of salts and the type of buffering agents used. Typically, the amount of Magnesium Sulphate that is added will exceed the saturation point, while the Sodium Chloride controls the polarity of the extraction solvent.

- Original Unbuffered Method: Magnesium Sulphate and Sodium Chloride
- AOAC 2007.01: Magnesium Sulphate and Sodium Acetate
- En 15662.2008: Magnesium Sulphate, Trisodium Citrate Dehydrate, and Disodium Hydrogen Sesquihydrate

Step 5: Extraction

- Once the extraction salts are added to the sample, the tube is shaken vigorously for 1 minute.

Note: Some pesticides, such as those that are strongly protonated at low pH, require control of the pH and thus will need to be buffered to pH 2-7 prior to extraction.

Step 6: Separation

- The tube is centrifuged to separate the organic layer from the sample at 4000 rpm for 10 mins.

Step 7: Decanting and Dispersive SPE (dSPE) Clean-Up

- An aliquot of the organic layer is decanted into a 15 mL centrifuge tube for clean-up by dSPE.
- The tube is shaken vigorously for 30 seconds.

NOTE: This step allows for the removal of matrix compounds (fats, proteins, chlorophyll, etc.) prior to analysis.

- Primary Secondary Amine (PSA) and Magnesium Sulphate are the most common clean-up used for the dSPE. For high-fat samples, C18 will be introduced into the dSPE, but for high-chlorophyll samples and carotenoids, Graphitized Carbon Black (GCB) is introduced into the dSPE.

Step 8: Separation

- The 15 ml tube is centrifuged to separate the supernatant from the dSPE media.

Step 9: Decant the Supernatant

- The organic layer is decanted and placed into a 2 mL LC sample vial.
- Depending on the detection scheme used and the desired reporting limits, a 2-10X concentration/dilution of the extract may be required to achieve the necessary sensitivity.

4.RESULTS

4.1. CHEMICAL ANALYSIS

Several chemical parameters were performed for pigeon pea such as moisture, ash, fat, acid insoluble ash, calcium, alcoholic acidity, fibre, protein carbohydrates and energy.

Table 4.1: Chemical Analysis of Pigeon Pea

PARAMETERS	PIGEON PEA
Moisture	9.84%
Ash	3.62%
Acid insoluble ash	0.22%
Calcium	131.24mg/100g
Alcoholic acidity	0.122%
Protein	21.67%
Fibre	2.45%
Fat	6.43%
Carbohydrate	58.44%
Energy	378.31 K/calories

4.2. SPECTROSCOPIC ANALYSIS

AAS ANALYSIS

Table 4.2.1: Determination of Trace Metals by Atomic Absorption Spectroscopy: Potassium

Serial number	Sample name	Concentration	Absorbance
	Blank		0
S1	Standard	1	0.001
S2	Standard	2	0.002
S3	Standard	3	0.004
S4	Standard	4	0.005
S5	Standard	5	0.007

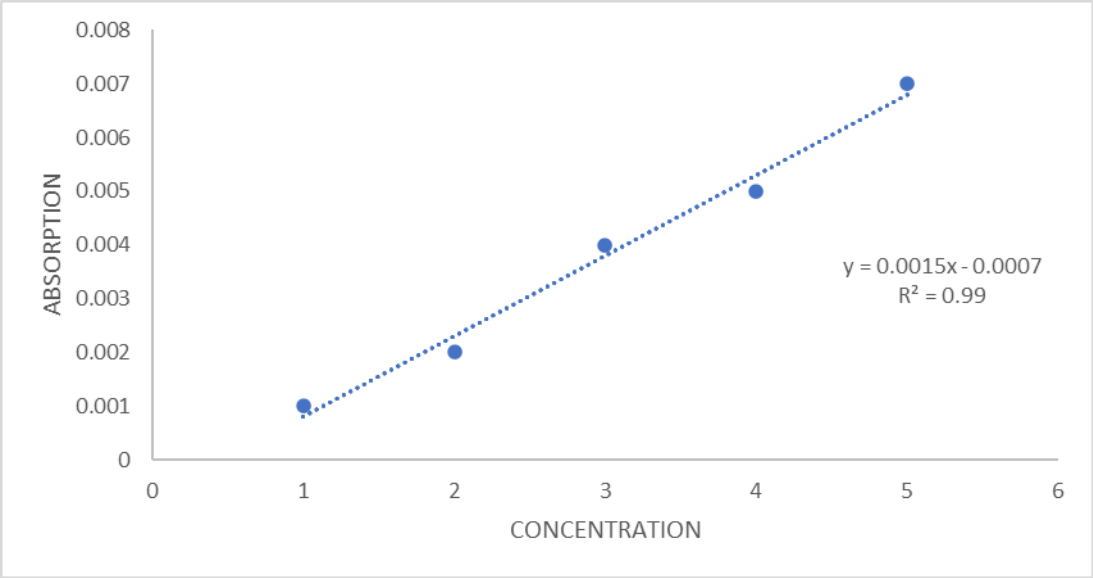

Figure 4.2.1: Graph showing standard curve of Potassium

Table 4.2.2: Determination of Trace Metals by Atomic Absorption Spectroscopy: Sodium

Serial Number	Sample name	Concentration	Absorbance
	Blank		0
S1	Standard	1	0.018
S2	Standard	2	0.03
S3	Standard	3	0.049
S4	Standard	4	0.07
S5	Standard	5	0.084

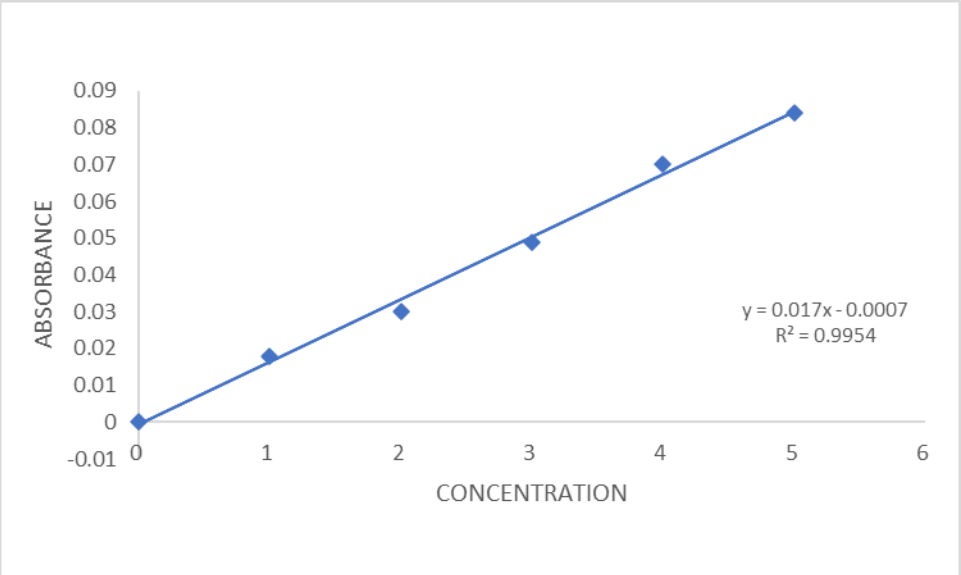

Figure 4.2.2: Graph showing standard curve of Sodium

Table 4.2.3: Determination of Trace Metals by Atomic Absorption Spectroscopy: Zinc

Serial Number	Sample name	Concentration	Absorbance
	Blank		0.001
S1	Standard	0.2	0.092
S2	Standard	0.4	0.183
S3	Standard	0.6	0.262
S4	Standard	0.8	0.333
S5	Standard	1	0.403

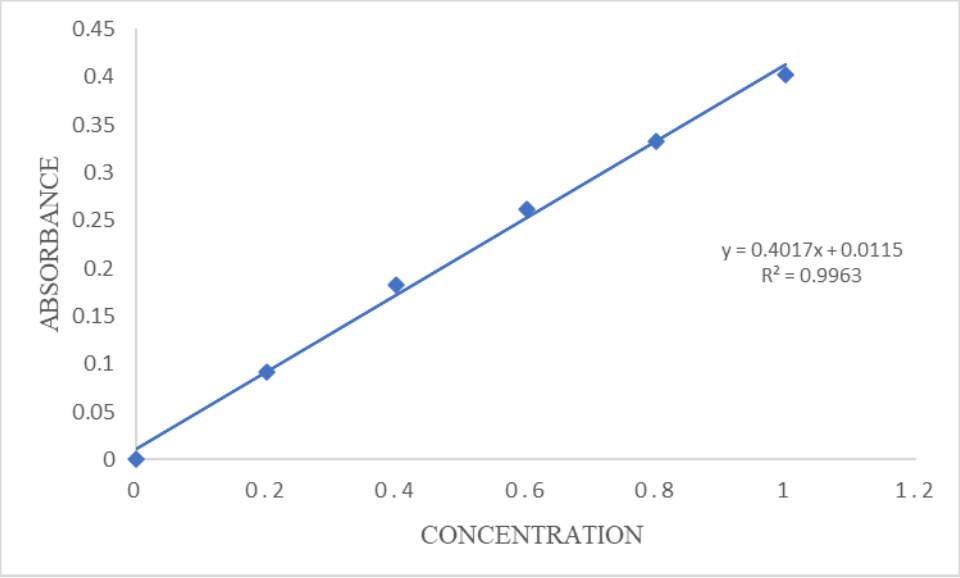

Figure 4.2.3: Graph showing standard curve of Zinc

Table 4.2.4: Determination of Trace Metals by Atomic Absorption Spectroscopy:

Copper

Serial Number	Sample name	Concentration	Absorbance
	Blank		0.004
S1	Standard	0.2	0.012
S2	Standard	0.4	0.022
S3	Standard	0.6	0.035
S4	Standard	0.8	0.043
S5	Standard	1	0.055

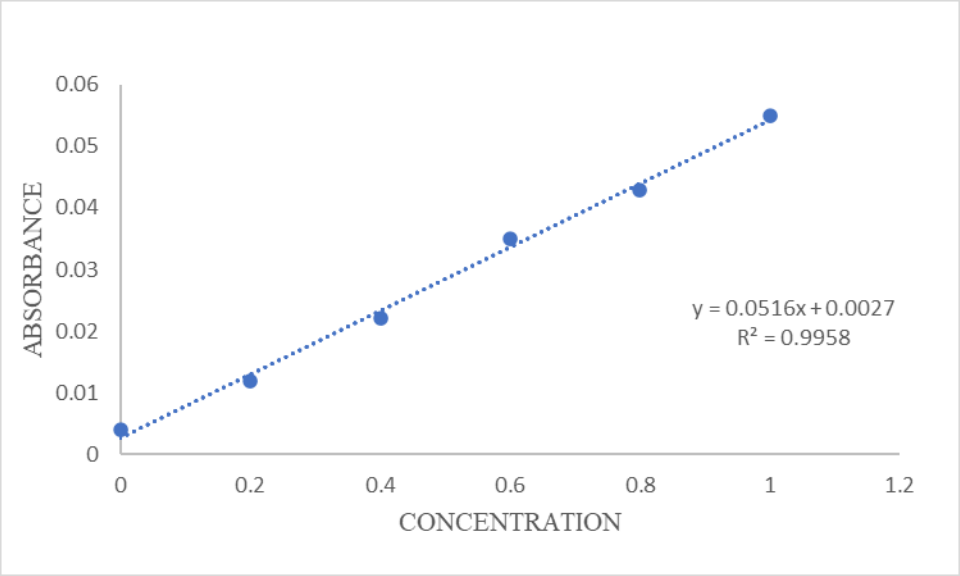

Figure 4.2.4: Graph showing standard curve of copper

Table 4.2.5: Determination of Trace Metals by Atomic Absorption Spectroscopy: Cadmium

Serial number	Sample name	Concentration	Absorbance
	Blank		0.003
S1	Standard	1	0.015
S2	Standard	2	0.045
S3	Standard	3	0.062
S4	Standard	4	0.089
S5	Standard	5	0.113

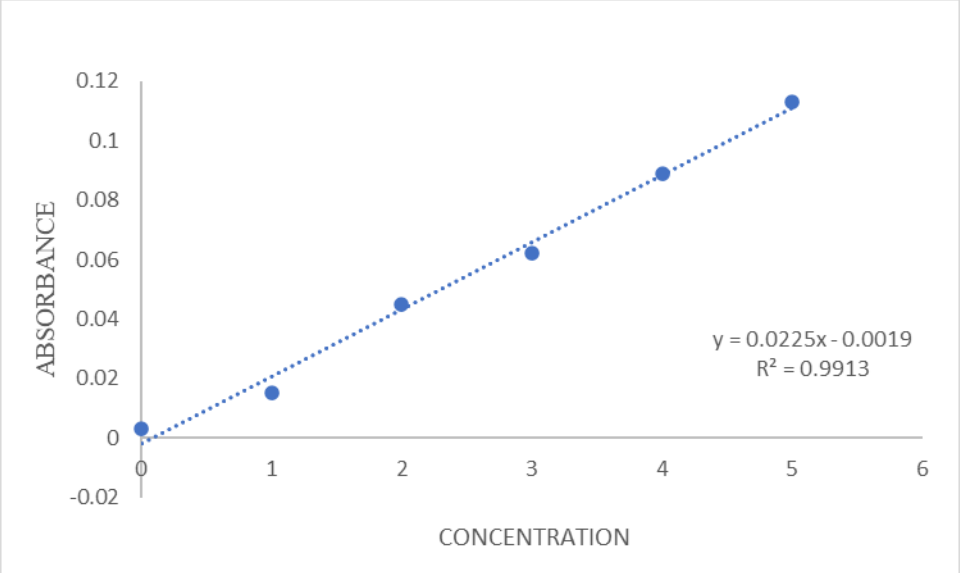

Figure 4.2.5: Graph showing standard curve of Cadmium

Table 4.2.6: Determination of Trace Metals by Atomic Absorption Spectroscopy: Calcium

Serial number	Sample name	Concentration	Absorbance
	Blank		0.001
S1	Standard	1	0.01
S2	Standard	2	0.019
S3	Standard	3	0.032
S4	Standard	4	0.043
S5	Standard	5	0.052

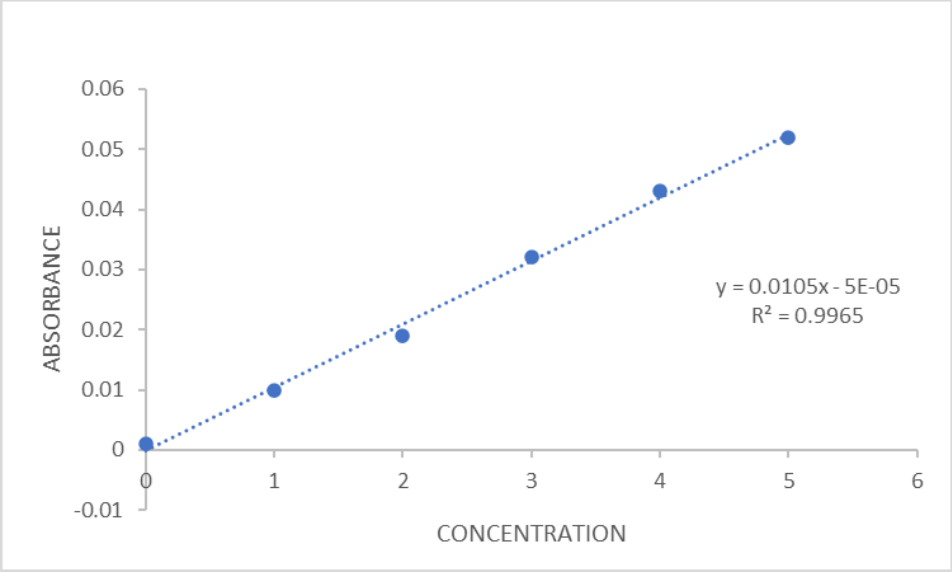

Figure 4.2.6: Graph showing standard curve of Calcium

Table 4.2.7: Determination of Trace Metals by Atomic Absorption Spectroscopy: Iron

Serial number	Sample name	Concentration	Absorbance
	Blank		0
S1	Standard	1	0.051
S2	Standard	2	0.072
S3	Standard	3	0.089
S4	Standard	4	0.115
S5	Standard	5	0.134

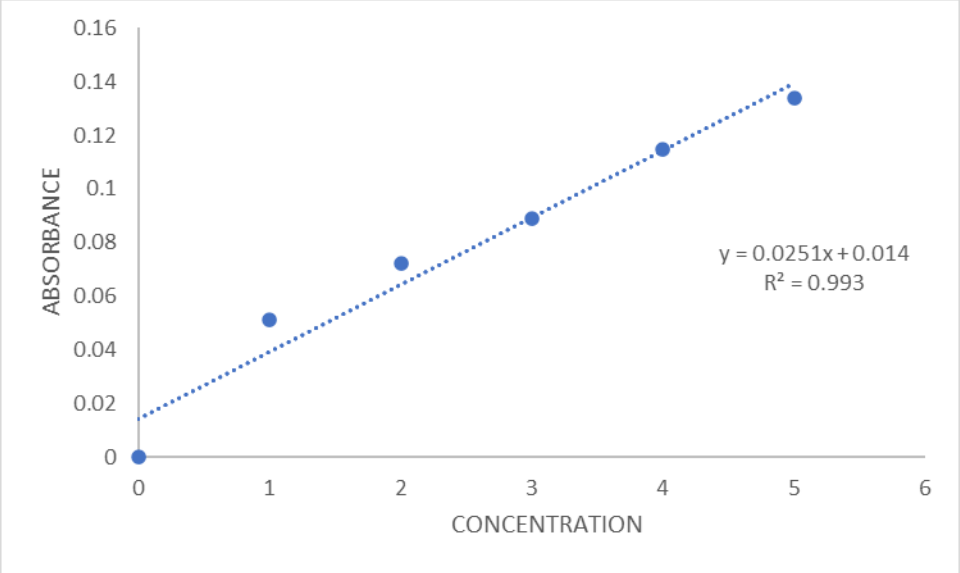

Figure 4.2.7: Graph showing standard curve of Iron

Concentration of different elements In Pigeon Pea was detected as follows

Table 4.2.8: Concentration of different elements in Pigeon Pea

Elements	Concentration (in ppm)	ABSORPTION
Potassium	4.207	0.046
Sodium	0.413	0.006
Calcium	2.993	0.018
Zinc	0.235	0.101
Cadmium	ND	
Copper	0.023	0.001
Iron	1.939	0.046

Table 4.2.9: Concentration of different elements in Chilka Daal

ELEMENTS	CONCENTRATION (PPM)	ABSORPTION
POTASSIUM	3.309	0.034
SODIUM	2.155	0.033
CALCIUM	4.467	0.048
ZINC	0.254	0.108
CADMIUM	ND	
COPPER	0.068	0.004
IRON	0.154	0.005

From the resultant concentration of the elements including minerals and heavy metals it was concluded that it can be used as a edible product.

Table 4.2.10: Determination of Total Phenol Content

TEST TUBES	GALLIC ACID SOLUTION IN ML	CONCENTRATION IN Mcg/ml	ABSORBANCE AT 650nm
S1	1.5	10	0.099
S2	3.75	25	0.245
S3	7.5	50	0.455
S4	11.25	75	0.785
S5	15	100	1.099
PIGEON PEA		UNKNOWN	0.4567

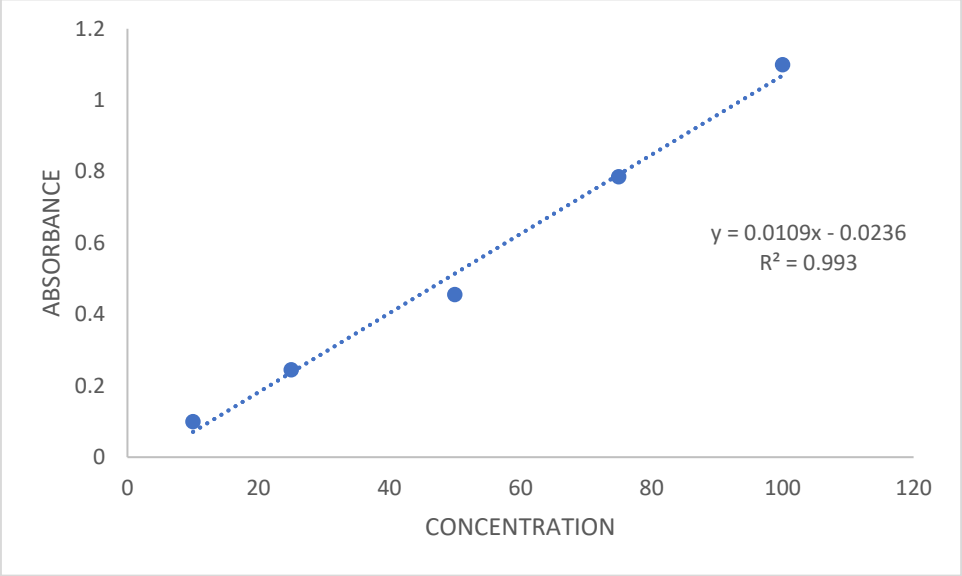

Figure 4.2.8: Graph showing calibration curve of Gallic Acid

CALCULATION OF UNKNOWN CONCENTRATION FROM CALIBRATION CURVE

Y = 0.0109x - .0236

(Y = mx + b),

x = Conc. of Gallic acid

Y = absorbance of sample

For pigeon pea Y = 0.4567, X = 44.04 mcg/ml

SAMPLE	TOTAL PHENOL CONTENT(TPC)(mg GAE/g) of extract
Pigeon Pea	87.9

Table 4.2.11: Determination of Total Flavonoid Content

TEST TUBES	Quercetin Solution in ml	Weight in Mcg/ml	Absorbance at 510nm
S1	1	20	0.227
S2	1	40	0.399
S3	1	60	0.667
S4	1	80	0.798
S5	1	100	0.996
PIGEON PEA	1	Unknown	0.2346

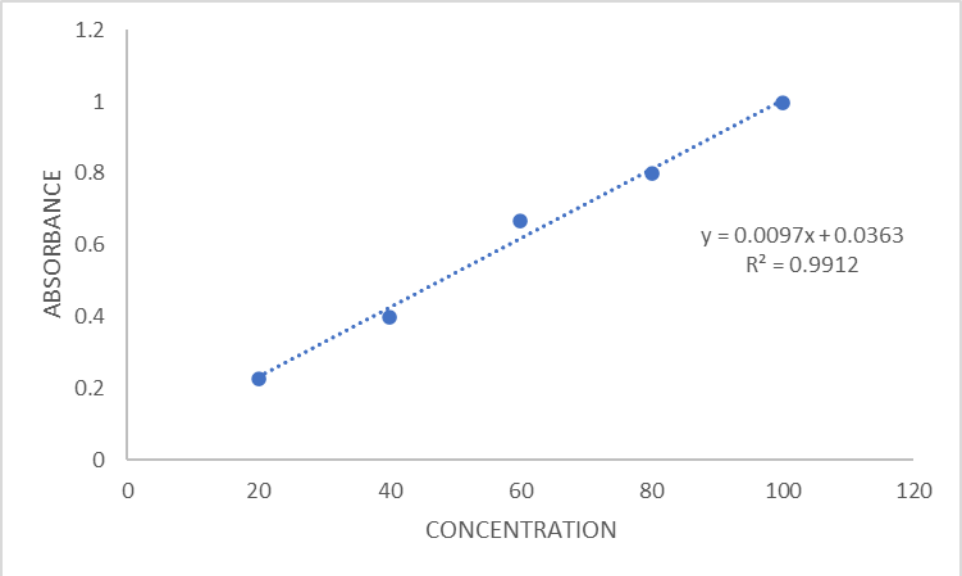

Figure 4.2.9: Graph showing calibration curve of Quercetin

CALCULATION OF UNKNOWN CONCENTRATION FROM CALIBRATION CURVE

Y = 0.0097x + .0363

(Y = mx + b),

x = Conc. of Quercetin

Y = absorbance of sample

For pigeon pea, y = .2346, x = 20.44mcg/ml

SAMPLE	TOTAL FLAVONOID CONTENT (TFC) (mg QE/g) of extract
Pigeon pea	40.80

Table 4.2.12: Pesticides Residue Result for Pigeon Pea:

S. No.	NAME	CATEGORY	MRL (mg/kg)	Results (mg/kg)
1	Acephate	Pigeon pea	0.03*	N.D
2	Ethion	Pigeon Pea	0.01	N.D
3	Flubendiamide	Pigeon pea	1.0	N.D
4	Indoxacarb	Pigeon pea	0.1	N.D
5	Lufenuron	Pigeon pea	0.01	N.D
6	Quinalphos	Pigeon pea	0.01	N.D
7	Thiophanate-Methyl	Pigeon pea	0.03*	N.D
8	Deltamethrin	Pigeon pea	0.01	N.D
9	lamda cyhalothrin	Pigeon pea	0.01	N.D
10	Methomyl	Pigeon pea	0.01	N.D
11	Spinosad	Pigeon pea	0.01	N.D
12	Thiodicarb	Pigeon pea	0.01	N.D

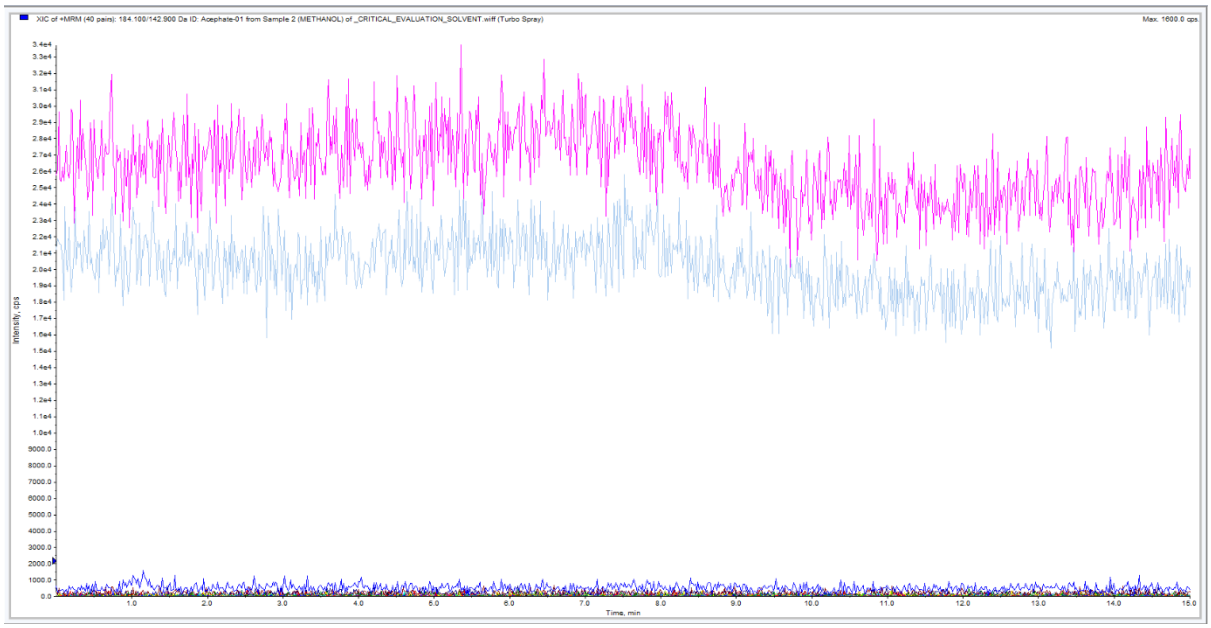

Figure 4.2.10: Chromatogram of Solvent

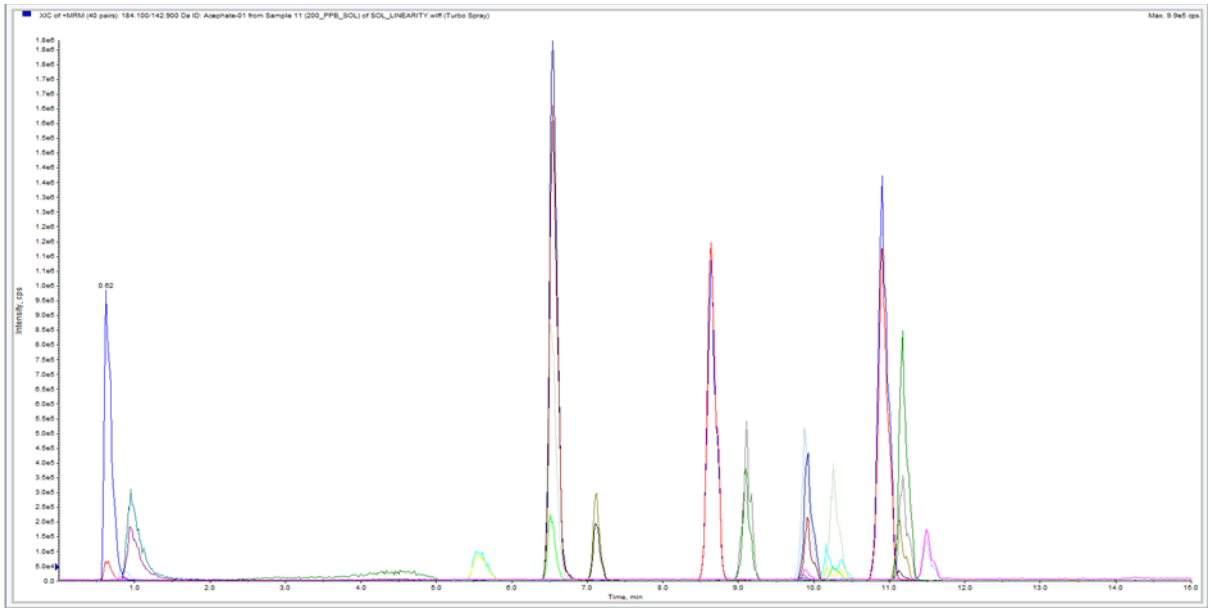

Figure 4.2.11: Chromatogram of Standards

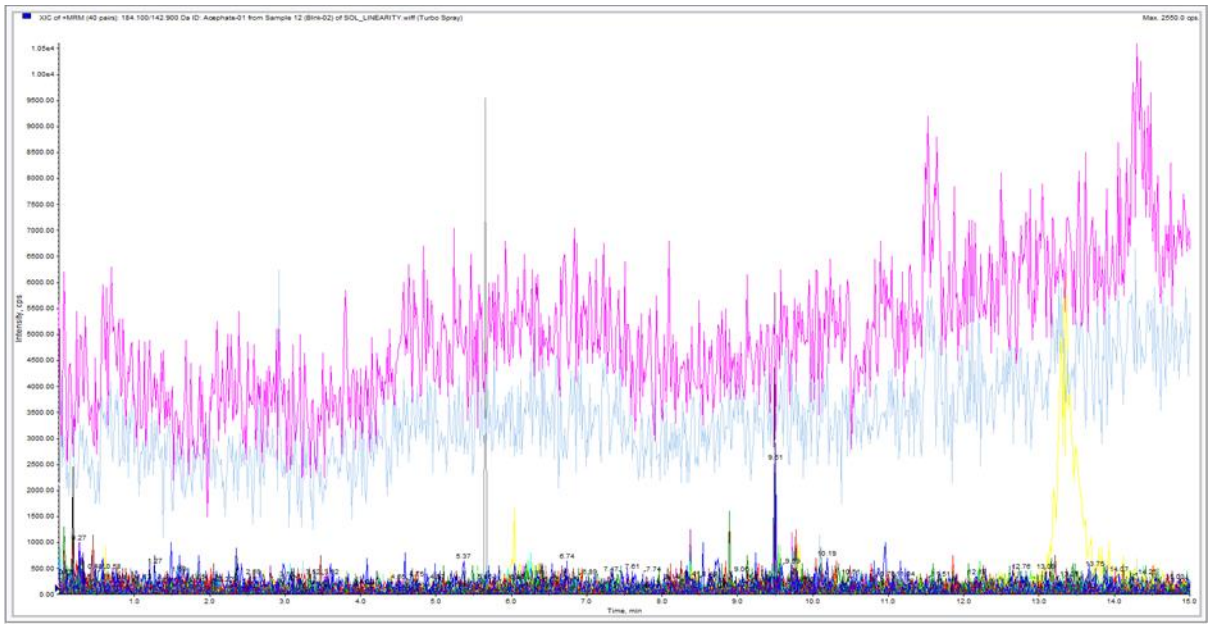

Figure 4.2.12: Chromatogram of Sample

5. CONCLUSION

In the present study moisture content, ash content, fat content, acid insoluble ash, alcoholic acidity, fibre, protein, carbohydrate, energy, trace metals, Ca, total phenolic content, total flavonoid contents were determined both chemically as well as spectroscopically as required, in pigeon pea., obtained from local market and found to be 9.845, 3.62%, 6.43%, 0.22%,0.122%, 2.45%, 21.67%, 58.44%, 378.31 k/calories, for cotyledons with seed coat potassium 4.207 pm, sodium 0.413ppm, zinc 0.235pm, copper 0.023pm, iron 1.139 ppm, for naked cotyledons potassium 3.309 ppm , sodium 2.155pm, calcium 4.467pm, zinc 0.254ppm, copper 0.068pm, iron 0.154pm and 131.24m/100g, 87.9 mg GAE/g , 40.80mg QE/g respectively. Trace metals were compared in both naked cotyledons as well as cotyledons with seed coat.

The analysis of pesticides residue in pigeon peas indicates that the majority of samples tested consistently showed non-detectable levels of pesticides. This result aligns with the principles of organic farming, which aims to minimize the use of synthetic chemicals in agricultural practices. The low or absent pesticide residues in organic pigeon peas demonstrate the effectiveness of organic farming methods, such as crop rotation, biological pest control, and natural inputs, in producing food that is safer for human consumption and the environment. These findings highlight the potential benefits of choosing organic pigeon peas as part of a healthy and sustainable diet.

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