

A STUDY ON *MANIHOT ESCULENTA* FOR BIOPESTICIDAL ACTIVITY AGAINST *ORYZAEPHILUS SURINAMENSIS*

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

By

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**DEPARTMENT OF BIOTECHNOLOGY
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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **P. Sravya (37230085)** and **M. Rogan (37230070)** who carried out the project entitled “**A STUDY ON MANIHOT ESCULENTA FOR BIOPESTICIDAL ACTIVITY AGAINST ORYZAEPHILUS SURNAMENSIS**” under our supervision from **December, 2020** to **April, 2021**.

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ABSTRACT

Biopesticides are derived from natural sources such as animals, plants, bacteria, and certain minerals. Now-a-day the use of chemical pesticides has led to the development of resistance in insects and also destroys crops. Biological pesticides have the advantage of providing novel modes of action against insects that can reduce risks of cross-resistance as well as offering new leaves for the design of target-specific molecules. This is because they are often most effectively used as a part of an integrated pest management approach. The present study is aimed to evaluate the biopesticidal activity of *Manihot esculenta* (Cassava) leaves to provide a much safer and effective alternative against this pest *Oryzaephilus surinamensis* (storage pest). Cassava belongs to the family Euphorbiaceae and is an annual crop of the tropical region in India. The qualitative phytochemical analysis and antimicrobial activity were performed for the different solvent crude extract of cassava leaves. The pesticide activity was tested against *Oryzaephilus surinamensis* (L.). In an *in vitro* condition. TLC was performed and GC-MS analysis was done to identify the active compound present in the methanolic crude extract. The GC-MS results showed that few compounds were found to be responsible for the pesticide activity. Molecular docking was performed for the compounds against the procathepsin L3 receptor.

Keywords: Biopesticides, *Manihot esculenta*, Pest, Cassava, Pesticidal activity, *Oryzaephilus surinamensis*, Antimicrobial activity, Molecular docking.

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LIST OF ABBREVIATIONS

SYMBOL	ABBREVIATION
μ	Micro
μm	Micrometer
μg	Micro gram
ANOVA	Analysis of Variance
cm	Centimeter
DPPH-	2, 2-diphenyl-1-Picrylhydrazyl
FeCl	Ferric chloride
FTIR	Fourier-transform infrared spectroscopy
Fig	Figure
g	Gram
GAE	Gallic acid equivalents
GC-MS	Gas chromatography massspectroscopy
HCl	Hydrochloric acid
hr	Hour
IC	Inhibiting concentration
Linn	Linnaeus
mins	Minute
ml	Milliliter
mM	MilliMolar
nm	Nanometer
PDA	Potato Dextrose Agar
ppm	Parts per million
Rf	Retention factor
TEAC	Trolox Equivalent antioxidant capacity
TLC	Thin-layer chromatography
UV	Ultraviolet
W/V	Weight / volume

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CHAPTER 1

INTRODUCTION

Over next 20 years, the need of agriculture has been of great importance in order to meet the rising needs of the human population (Chandler et al., 2011). All living organisms are subjected to predation, parasitism or competition from other organisms. This study of interactions has led to the identification of many potential opportunities to the use of living organisms to protect against pests, fungal, bacterial and viral diseases, weeds, nematodes and mollusk pest (Rodgers, 1993). Indian agricultural is that the backbone of our economy, India's crop yield prospects suffers loss at a rate of 30 million tons/ year. The major reason for such shortage was the crop or yield loss incurred by various insect pests. Agricultural fields has been facing innumerable destructive activities of pest like fungi, insects and weeds from time immemorial, which is leads to the rapid depletion of yields. With the rise of chemical pesticides, this kind of crisis was resolved to a greater extent. But due to over dependence on chemical pesticides and eventual uninhibited use of them has entailed for alternatives mainly for environmental concerns (Gupta and Dikshit, 2010). There are estimated to be about 67,000 different species of pest that cause about 40 percent reduction in crop yield which in turn undermine food production among other things such as poor soil, inclement weather and farmers limited access to technical knowledge. These crisis was resolved to greater extend with the use of chemical pesticides. The chemical used to control the pests have led to degraded soils and unwanted pollution which in turn ensued nutritionally imbalanced and unproductive lands. This risk hazard also raised many food safeties concerns among the domestic consumers and also posed a threat to exported crops (Gupta and Dikshit, 2010).

Grain storage is a key household food security that is practiced by the small holder farmers but the storage insect pests are a major problem which is causing damage and economic losses. Additionally, the climate change (cc) is another factor expected to negatively affect grain storage in India, a region that is highly vulnerable to the effects of climate change (Boko et al., 2007; Tanya et al., 2013). Global warming is likely to affect the populations of the stored product insect pests,

increase degradation rates of storage insecticides and reduce the effectiveness of storage technologies such as hermetic bags (Delcour et al., 2014).

Furthermore, one of the main reasons for the concern of overuse of pesticides is due to the development of pesticides resistance in prominent pests and the remaining of pesticide residue in dairy and agricultural products. Studies show that one of the main reasons for pesticide resistance is due to the extensive use of pesticides on pests and its host plants. Botanical insecticides are the best alternatives to synthetic chemicals which can be safely used to control insect pest populations without harmful effects on the environment and users. Unlike synthetic insecticides, the natural compounds are safe for natural enemies of insect pests (Isman, 2006).

Therefore, it had become imperative to find an eco-friendly alternative to overcome the drawbacks associated with synthetic pesticides. The use of biopesticides has offered an ecologically sound and effective solution to pest problems and provide a better solution than chemical pesticides (Salma and Jogen, 2011).

1.1. PESTICIDE

The development of world agriculture, trade, and mechanization leads to even more extensive monocultures and consequently larger stores has worsened the problems with insect pest. Conferring on to a study conducted by the International Food Policy Research Institute, about RM184.9 billion worth of crop production was destroyed every year by pest in Asia and stored product insects (SPI) have been found as one of the pests that contributed to the losses. It causes damage to stored products like grains by directly feeding on the grain at some stages in their life cycle (Nurul Huda and Noor Amni, 2019).

A certain upsurge in the global demand for food in the next few years is looming owing to incessant population and growth of consumption. The last five years has experienced impressive growth in food production, associated in a marked decline in the percentage of the hungry people, globally, despite a double increase in the total population. The natural antioxidant content of food products has mainly focused on most of the studies in recent years owing to its involvement in defense against diseases (Folayemi Janet Isaac-Bamgboye *et al*, 2020).

Conservation of reserved food grain stocks is necessary to ensure a continuous supply at stable prices. Stored product pests are one of the important pests after harvesting in grain storage. These pests with feeding and contaminating with excrete can cause damage to the store products. Among these pests, coleopteran stored pests are the fiercest pests that are repeatedly reproductive and extremely high generation in optimal conditions (Mahdi and Behnam, 2016). Insect pest infestation is a main problem in the storage of cereals and pulses. Realizing that the confused application of synthetic pesticides has created major challenges to man and ecosystem, there is a global report on to contain pests using non-chemical methods, particularly to reduce the pesticide residues in food (Singh & Huidrom, 2013).

1.2. BIOPESTICIDES

Biopesticides are developed from naturally occurring living organisms such as animals, plants, and microorganisms (eg. bacteria, fungi, and viruses) that can control serious plant- damaging insect pests by their nontoxic eco- friendly mode of action on pests. They have gained more importance on pests as they pose less threat to the environment (Saxena & pandey, 2001). One of the greatest advantages of biopesticides are:

1. They are characteristically less harmful than chemical pesticides.
2. They are target-specific and so they only attack the target pests unlike, chemical pesticides which also cause adverse effects on non-target organisms.
3. They are effective in small doses.
4. Unlike chemical pesticides, they are bio-degradable and hence, they don't leave any harmful residues in the environment.

When biopesticides are used as a constituent of pest management, its efficiency can be equal to that of the synthetic pesticides, particularly for crops like fruits, vegetables, nuts and flowers. They also overcome the concern of residue problems especially for consumers of edible fruits and vegetables (Kumar, 2012).

Biopesticides have been gaining importance with those concerned with developing environmentally friendly and safe integrated crop management (ICM)- compatible approaches and tactics for pest management. They have also gained attention in farmer's recent trend of "Organically produced food" and the more effective introduction of "Biologically based products" (Copping & Menn, 2000).

1.3. TYPES OF BIOPESTICIDES

Biopesticides mainly fall into three categories based on the active substances:

1. Microbial biopesticides
2. Biochemical biopesticides
3. Plant biopesticides / botanical pesticides

1.3.1. Microbial biopesticides

They contain a microorganism (bacterium, fungus, virus, protozoan or alga) as the active ingredient which are used for the biological control of plant pathogens such as pests, insects and weeds. Different microbial pesticides are responsible for control of different kinds of pests because each separate active ingredient is relatively specific for its target pests. For instance, certain type of fungi controls weeds and other types that kill specific insects (Gupta & Dikshit, 2010). The most widely used organism in the development of microbial pesticides is varieties of insect pathogenic bacterium *Bacillus thuringiensis*, or Bt. They produce a specific protein during the spore formation of the bacterium that is harmful to specific insects such as Lepidoptera, coleopteran and dipteral (Chandler et al, 2011).

1.3.2. Biochemical pesticides

Biochemical pesticides are naturally occurring substances that control pests by non- toxic mechanisms. These types of pesticides include substance that interfere with growth or mating, that such as plant growth regulators, or substances that repel or attract pests, such as pheromones. Pheromones are certain chemicals produced by insects which are stimulated due to a certain behavioral reaction from other individuals. They can be known as sex pheromones, aggregation pheromones, alarm pheromones, etc., according to type of effects it displays. They

have become essential tools in monitoring and managing agricultural pest populations (Witzgall et al., 2004). Therefore, Environmental protection Agency (EPA) has established a committee to determine whether a pesticide meets the specified criteria for a biochemical pesticide (Salma &Jogen, 2011).

1.3.3. Plant Biopesticides

Plant biopesticides or Botanical pesticides are pesticidal substances that use the natural compounds from plants from plant to give security to insect pests. Exploring the potential to utilize the properties of plants has become a key focus in pest control. Some plants are known bioactive metabolites, which show repellent and toxic effects on a wide ran pest. These compounds were initially used in the nineteenth century and by the early period of the twentieth century, their use and benefits have been established worldwide (Morgan, 2004). Over the years, there are found to be more than6000 plant species that have been screened for insecticidal activity and nearly 2500 species belonging to 235 plant families were found to possess considerable pest control activity. Various isolated compounds from plants indicate that approximately 350 compounds are insecticidal (Saxena, 1998). Therefore, some plants also act as repellents and when effective, it causes the target pest to make an oriented movement away from the source of stimulus and in case of no escape, it leads to the death of the pest (Owusu, 2001).

1.4. MANIHOT ESCULENTA CRANTZ

TAXONOMIC TREE

Domain	: Eukaryote
Kingdom	: Plantae
Phylum	: Tracheophyta
Class	: Dicotyledons
Order	: Malpighiales
Family	: Euphorbiaceae
Subfamily	: Crotonoideae
Genus	: <i>Manihot</i>
Species	: <i>M. esculenta</i>



Fig: 1.1: *Manihot esculenta* (Cassava leaves)

Cassava (*Manihot esculenta* Crantz) is consumed all over the world as one of the leading origin of carbohydrates and as an essential food. The capacity of cassava plants to grow on lands with less water supply, fertilizers and agrochemical inputs allows simpler, cost-effective cultivation and management of this plant (Idris, S *et al*, 2020). Cassava plant is also called life blood because of the consumption of its starchy tubers as staple starch, textile and pharmaceutical applications. Cassava is a potential origin of proteins, antioxidant constituents, vitamins B1, B2 and C and minerals, iron, zinc, manganese, magnesium and calcium. It is also rich in micro and macronutrients. Certainly, Cassava manufacture has been increased worldwide because of different industrial applications such as healthy food for human, animal feed, raw material and also medicinal applications like antidiabetic, anticarcinogen, antioxidant and antitumor potential. Cassava is considered a precious plant including industrial and therapeutic potential, cassava in terms of the ethanolic and aqueous extracts involve alkaloids, anthraquinone, flavonoids, anthocyanosides, tannins and Saponins (Nermeen *et al*, 2016).

They contain cyanoglycosides, linamarin, lotaustralin and methyl linamarin. Its leaves are used to heal some diseases which include measles, small pox, chicken pox, skin rashes and leaf sap latex used for eye condition, used for rheumatism, fresh rhizome applied to ulcers, leaves used for fevers and headaches, colds, fever and to treat constipation, used in the treatment of ringworm, tumor, conjunctivitis, sores and abscesses. Amino acid which is present in cassava leaves are also useful for wound healing in the skin, replacing damaged cells, bone health, good for

memories and helps body metabolic system. Chlorophyll in the leaves contains antioxidants and anticancer agent. Cassava leaves provides more benefits for our health. So, our health can be supplemented with the intake of cassava leaves (Mity Thambi and Tom Cherian, 2015).

The tuber is commonly used for the production of tapioca chip and starch. Starch is used generally for the manufacture of monosodium glutamate, glucose and paper products. Tubers are also prepared as feed for livestock. New food uses of cassava incorporate flour in gluten free or gluten-reduced products (Quartey *et al*, 2016). The aerial part of cassava plant treated as an agricultural by-product, but that nutritionally, presents great potential for human and animal consumption, has been gaining prominence. Moreover, their use can be responsible for an extra income to various producers that live on the cassava culture. Those leaves are rich in proteins and vitamins A and C, and minerals, especially Mg, Fe, Zn and Mn, obtained at a low cost, when compared to conventional leafy vegetables (Kay Zar Linn and Phyu Myint, 2018).

Kasoami made from cassava with the Latin name *Manihot esculenta Crantz*, also known as the Latin name *Manihot utilissima*, contains flavonoids and polyphenols in the stems tubers. Flavonoids and polyphenols are main source of antioxidants. Antioxidants are compounds that can inhibit the oxidation process. This oxidation process is a chemical process that produces free radicals, which cause a chain reaction that can damage the cells of organisms. Oxidizing substances can be in the form of ROS (reactive oxygen species) which are naturally produced by the body because of the metabolic waste, which can be harmful to the body (Haidir Amin *et al*, 2020).

The natural dietary antioxidant content in food has been focus of much investigation recently because its contribution in protection against diseases. Unfortunately in the Far North Region of Cameroon, the cultivation of cassava is almost unknown or limited in certain villages because it is not one of the stable foods (Benoit *et al*, 2015).

In addition to the application of cassava tubers as a staple food, cassava leaves are also used by ordinary people to overcome and treat ulcer disease, rheumatism, gout, diarrhea, fever, headache, night blindness, intestinal worms, and beri-beri disease (Resmi *et al*, 2020). Cassava will be affected by fungi, bacteria, viral and virus-like and mycoplasma agent. Accomplishment of bio-control measure through pathogens which occurs naturally in the field has been made a reality via antibiotics, bacteriocins and bacteriophage. The anti-microbial properties of microbes have consequently led to the classification of different anti-microbial substances as organics acids (lactic acid and formic acids), diacetyl, and hydrogen peroxide alone or in combination (Agarry *et al*, 2020).

1.5. ORYZAEPHILUS SURINAMENSIS

Taxonomic Tree

Domain : Eukaryota
Kingdom : Metazoa
Phylum : Arthropoda
Subphylum : Uniramia
Class : Insecta
Order : Coleoptera
Suborder : Cucujoidea
Family : Silvanidae
Genus : *Oryzaephilus*
Species : *O. surinamensis*



Fig: 1.2: *Oryzaephilus surinamensis* - Adult

Globally, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) are the important stored-grain pests, damaging 10 to 40% of all stored agricultural crops. These beetles feed on grains, dried fruits, flour, sugar, candies, tobacco, and many other plant products intended for human consumption, and also dried meat. Recently, the intense use of chemicals for the control of stored products pests has developed in serious problems, which includes pest resistance to insecticides, environmental impurity, the presence of unacceptable pesticide remains in foods, and lethal effects on non-target organisms. Botanical insecticides have been used long as options to synthetic insecticides for insect pest management due to its botanical pose little trouble to the environment or to human health (Fatehia Gharsan *et al*, 2018).

Oryzaephilus surinamensis one of the serious pests which have been used in many kinds of food like dry fruits, spices, nuts, cereal and other cereal products. *Oryzaephilus surinamensis* a Coleopteran of family Silvanidae also called as saw toothed grain beetle/ flat grain beetle as it is dorsa-ventrally flattened body consisting of saw like projections on the thorax region. Stored grain infestation generally occurs from top to bottom or vice versa through migration of pest *Sitophilus oryzae*, *Oryzaephilus surinamensis* and *Tribolium castaneum* called as vertical infestation. Synthetic insecticides like organ chlorides, organophosphorous, carbamates and synthetic pyrethroids commonly used to control stored grain pests but this reduces quality of the grain, creates smelly odor, hazardous to human health causing bio-magnification (Neetu Kachhwaha *et al*, 2015).

Among all stored product pests, the sawtoothed grain beetle (*Oryzaephilus surinamensis*) is most commonly used pest all over the world and its infestation can originate at the manufacturing, storage or retail levels. The distribution of the beetle is significantly damaged by various factors like food availability, processing practices, temperature conditions in various areas, and interaction between species. Foods that may be infested include cereals, flours, pastas, dried fruits, nuts, dried meats, candies, and other similar packaged goods (Nurul Huda and Noor Amni, 2019).

Moreover, cassava would contribute to agricultural industrial development. Hence, its starch shows potential for the enhancement of products with high added value in alimentary and non-alimentary industrial applications such as pharmaceutical products, paper, fabrics, sweeteners, animal feed, biofuels and even the manufacture of bioplastics (Jose *et al*, 2020).

Given this background knowledge, the present study is aimed to investigate the phytochemical content, antioxidant activity, antimicrobial activity on Cassava (*Manihot esculenta* Crantz) and insecticidal property of methanol, ethyl acetate and chloroform extracts of *Manihot esculenta* for controlling *Oryzaephilus surinamensis* was studied.

CHAPTER 2

REVIEW OF LITERATURE

2.1. HISTORY OF *MANIHOT ESCULENTA*

Cassava (*Manihot esculenta*) is a precious plant used in industrial and therapeutic potential; which is rich in essential nutrients for the body such as carbohydrates, vitamin A, vitamin C, iron, calcium, phosphorus and phenolic compounds. Present study is to evaluate the efficiency of absolute organic solvent (methanol) and inorganic solvent (water) for phenols extraction from stem calli of Cassava and identification of the phenolic compounds of their crude extracts (methanol and aqueous extracts) by HPLC. To study the cytotoxicity of both methanol and aqueous extracts against breast cancer cell lines (MCF7) were determined. Phenolic acid, flavonoids and cinnamic acid derivatives were found in methanol and aqueous extracts by HPLC at 280, 320 and 360 nm of the UV chromatograms. This work was performed by DNA fragmentation and detection DNA content using flow cytometry of DNA in different groups. The results of DNA electrophoresis demonstrated that DNA damage observed in breast cancer cell line intoxicated has been found with cassava aqueous extract protection and the treated MCF7 showed a single diploid peak (Nermeen *et al*, 2016).

2.2. HISTORY OF *ORYZAEPHILUS SURINAMENSIS*

The biological study by host range or food preferences of *O. surinamensis* important for the development of sustainable management practice to control its infestation. This study was to discover the food preference of *O. surinamensis* different plant products in relation to food type and moisture content. Twenty adult of *O. surinamensis* were exposed to three various group of food; dried fruits (date, raisin, apricot, fig), grain/cereals (rice, barley, oat grout, dried maize), and nuts (almond, ground nut, walnut, cashew nut) for 240 h in laboratory Kulliyyah of Science, IIUM Kuantan. Moisture content in each food was also studied. Further analysis on food moisture suggested that under current experimental conditions (temperature of 27°C and 64% relative humidity within 240 h of exposure), food moisture content does not affect pest infestation and distribution (Nurul Huda and Noor Amni, 2019).

2.3. CARBOHYDRATES IN CASSAVA

Cassava (*Manihot esculenta* Crantz) is one of the major sources of carbohydrate. Its root is used to produce various foods such as cassava chips, while the other parts are potentially to produce feeds and other useful products. This research was to evaluate the composition of each part in the cassava plant and characterized using Fourier-transform infrared spectroscopy (FTIR) and the thermal properties using thermo gravimetric analyzer (TGA). The results obtained showed that the portions of fresh cassava plants namely flesh; stem, peel, discarded tuber and the leaf were recorded at 50.06%, 31.01%, 10.63%, 6.92% and 1.49% (w/w), respectively. Meanwhile, cassava flesh also has potential as an animal feed since it has low crude fiber but high in carbohydrate and gross energy content. FTIR spectra showed that there were different functional groups present in the samples. From the TGA data, it showed that the major components in samples were cellulose which started to decompose rapidly at maximum degradation rate temperature of 315-400°C. Various parts of cassava plants are wanted to help the agriculture industry in producing alternative animal feed at the same time minimizing the impact of waste generated in the environment (Idris *et al*, 2020).

Essential oils from aromatic plants are recognized as proper alternatives to chemical fumigants. In this research fumigant toxicity, repellent activity and persistency of Citrus leaves *Citrus aurantium*. essential oil were tested against three stored product pests, the sawtoothed grain beetle, *Oryzaephilus surinamensis*(F.), cigarette beetle *Lasioderma serricorne*(L.) and the rice weevil, *Sitophilus oryzae*(L.). The essential oils were extracted from citrus dried leaves by Clevenger-type distiller. Repellency effect of the essential oil was evaluated by comparing the number of adults in both treated and untreated portions. Results showed that the mortality increased with increasing concentration. The LC50 values were 64.94, 202.49 and 364.25 and the LC90 were 144.94, 659.29 and 663.05 µl/l air respectively. Toxicity of oil against *O.surinamensis* was higher than two other pests. The highest and lowest repellency effects of four concentrations (0.1, 0.2, 0.41 and 0.83 µl/cm²) were 96.6% and 73.33% for *O.surinamensis* and *L.serricorne* respectively. The results have shown

that the fumigant toxicity of *C.aurantium* on these stored pests were remarkable, therefore this oil could be a safer fumigant to control these pests than those currently used (Mahdi Kabiri Raeis Abad and Behnam Amiri Besheli, 2016).

2.4. CASSAVA LEAVES AGAINST PESTS

Manihot esculenta, Cassava also called, tapioca-root, kassa (predominantly in India) a woody shrub of the *Euphorbiaceae* family native to South America. In the current study reveals that, plant extracts of *Manihot esculenta* leaves in ethyl acetate solvent is highly toxic against adults of *Sitophilus oryzae*. The ethyl acetate extract of *Manihot esculenta* would be used as a potential grain protectant against *Sitophilus oryzae*. The use of botanical materials as insecticides will benefit our agricultural sector. Therefore, the findings of the current experiments strongly support the use and exploration of botanicals in pest management practices (Mity Thambi and Tom Cherian, 2015).

In this research *Manihot esculenta* Crantz. (Cassava) was studied to find out the types of organic constituents present in the samples, preliminary phytochemical investigation was carried out by test tube and TLC screening methods. The nutritional values such as protein, fiber, fat and carbohydrates were also studied by the AOAC (Association of Official Agricultural Chemists) method. The total phenolic content of various extracts was determined by the Folin-Ciocalteu method. *In vitro* antioxidant activity was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Preliminary phytochemical tests have revealed that the presence of alkaloids, α -amino acid, carbohydrates, glycosides, tannins, phenolic compounds, cyanogenic glycosides, saponins, starch, steroids, terpenoids, flavonoids, and reducing sugar in the sample. Proximate composition of Cassava leaf has been found as 10.70 % of moisture, 3.19 % of crude fat, 29.28 % of protein, 16.25 % of dietary fiber, 33.71 % of carbohydrate, 6.87 % ash and 279 kcal /100 g based on dry sample. The higher extractable total phenolic was recorded in 70 % ethanol extract (748.22 μ g GAE/mg) followed by aqueous extract (446.22 μ g GAE/mg) for the leaf sample. Both 70 % ethanol and aqueous extracts showed significant DPPH radical scavenging activity. Ethanol extract of (IC₅₀ = 17.69 μ g/mL) leaf showed higher level of free radical scavenging activity and found to be the lower in aqueous extract of

leaf ($IC_{50} = 42.64 \mu\text{g/mL}$). From these results, it can be inferred that this leaf possesses nutrients and antioxidant properties and has nutraceuticals potential for the treatment of malnutrition. (Kay Zar Linn and Phyu Myint, 2018).

2.5. PHYSIOCHEMICAL AND ANTI-OXIDANT PROPERTIES OF CASSAVA

Leaves from five cassava (*Manihot esculenta*) genotypes including local and improved were evaluated for their physicochemical and antioxidant properties. Results showed significant ($p < 0.05$) variations with the different analyzed parameters. Leaves from the improved genotype 92/0326 exhibited the highest ash content (2.34 mg/g fresh weight). Mineral elements were more present in *IRAD4115* and 92/0326 cassava leaves. Leaves from *IRAQ 4115* exhibited the highest carbohydrates content meanwhile those from local genotype *EN* presented a high protein content (6.64%). The improved variety *IRAD4115* has the highest total phenolic compounds as well as ascorbic acid (40.51 $\mu\text{g/g}$) and the highest total antioxidant activity. Cassava leaves are a very important source of macronutrient; micronutrient minerals and antioxidant compounds that can contribute in the strive against chronic malnutrition leading to a better state of health in rural and urban populations (Benoit *et al*, 2015).

The chemical composition of plants used in traditional medicine exhibits biologically active compounds, such as tannins, flavonoids, and alkaloids and becomes a promising approach to treat microbial infections, mainly with drug resistant bacteria. The aim of the present study was to evaluate the hydroethanolic leaf extracts of *Tamarindus indica* (tamarind) and *Manihot esculenta* (cassava) as antimicrobial potential against *Pseudomonas aeruginosa* clinical isolated and Methicillin-resistant *Staphylococcus aureus*. Hydroethanolic leaf extracts were prepared and characterized by high-performance liquid chromatography/diode array detection, Fourier transform infrared, 1,1-diphenyl-2-picrylhydrazyl, and ultraviolet visible methods. The antimicrobial activity against four strains of clinical relevance was evaluated by the micro dilution method at minimum inhibitory concentrations. Phenolic compounds such as flavonoids were detected in the plant extracts. Our results suggested that polyphenols and flavonoids present in *T.indica* leaf extracts are a potential source of antimicrobial compound. The *T.indica* extract showed

antibacterial activity against *S.aureus* and *P.aeruginosa* while *M.esculenta* had effect only on *P.aeruginosa* meropenem resistant (Zenon Machado Lima *et al*, 2017).

Plants contain diverse phytochemicals with different solubility levels, depending on their molecular charges and polarity. This study was conducted to examine the effects of three extraction solvents in their pure and aqueous forms: absolute petroleum ether, acetone and ethanol on the phytochemical profile and antioxidant activities of *Manihot esculenta* leaves extracts. The preliminary phytochemical investigations were performed, using standard procedures. The in vitro antioxidant properties were estimated by: 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH), Hydrogen peroxide (H₂O₂) scavenging activity, Ferric Reducing Antioxidant Power (FRAP) and Ferric Thiocyanate (FTC). The phytochemical analyses revealed the occurrence of an array of compounds: alkaloids, flavonoids, cardiac glycosides, tannin phenols, saponins and anthraquinones, the concentration of which varied with the solvent type. A considerable presence of all phytochemicals was found in the aqueous ethanol. The extracts from pure solvents were much less effective against DPPH synthetic radical than those 50% diluted. The petroleum ether extract provided the least antiradical activity, while the aqueous ethanol was the richest. Thus, the results of antioxidant activity positively correlated with the phytochemical results, with the aqueous ethanol showing the maximum activity overall. The results indicated that the extraction solvents considerably affected the phytochemical contents and the antioxidant activities of the tested extracts. These extracts can potentially serve as the alternative sources of natural antioxidant preparations (Israel, 2020).

2.6. CYTO-TOXICITY TEST AGAINST *ORYZAEPHILUS SURINAMENSIS*

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) cause extensive damage to many stored products, thereby reducing their nutritional and economic value. In this study was to identify the toxicity of 5 plant oils isolated from lavender (*Lavandula angustifolia* Mill, *Lamiaceae*), onion (*Allium cepa* L. *Amaryllidaceae*), flax (*Linum usitatissimum* Mill, *Linaceae*), caraway (*Carum carvi* [Lindl.] H. Wolff, *Apiaceae*), and brown galingale (*Cyperus fuscus*, or *saad* L., *Cyperaceae*) for use on the stored-products insect pests *T.castaneum* and *O.surinamensis*. The efficacy of these oils was evaluated at

concentrations of 1, 2, 3, and 4 μL per mL and at an exposure time of 24 h. Results indicated that *O.surinamensis* was more susceptible to plant oils than *T.castaneum*, which showed a greater resistance to these natural products. Results also revealed that caraway and lavender oils were the most toxic of all treatments rendered for *T. castaneum*, with 50% lethal concentration (LC50) values of 1.2 and 2.4 μL per mL, respectively. The onion and lavender oils displayed the highest efficacy on *O. surinamensis* with insect mortality reaching 100% with onion oil and an LC50 value for lavender oil of 0.6 μL per mL. These results suggest that onion, lavender, and caraway oils are potentially promising and environmentally acceptable alternatives for the control of *T.castaneum* and *O.surinamensis* (Fatehia Gharsan *et al*, 2018).

2.7. MANIHOT ESCULENTA AGAINST STORED PESTS

Fumigant toxicity of insecticidal principles extracted from tuber rind, fresh leaf, fresh leaf with petiole, and dried leaf of cassava (var. M4) was studied against four major stored-product insect pests *viz.* *Sitophilus oryzae* (L.), *Rhyzopertha dominica*(F.), *Tribolium castaneum*(Herbst) and *Callosobruchus chinensis*(L.) under laboratory conditions. Mortality of the test insects varied with respect to extracts collected from different parts of the plant, and time of exposure. *Callosobruchus chinensis* was highly susceptible and showed immediate knockdown effect to the active principles extracted from tuber rind, fresh leaf, fresh leaf with petiole, twig and semi-dried leaf. Mortality of *S.oryzae* was 100% at 1 HAT with tuber rind extract, but no response was observed from the extract collected from semi-dried leaf, twig, and leaf with petiole. No fumigant action was observed in all the four coleopteran pests exposed to the extract collected from dried leaves. The study showed that fresh leaf and tuber rind are good sources for the extraction of biofumigant against major coleopteran pests; however dried leaves are unfit for same purpose (Arumughan Jayaprakas Cheruvan and Ragesh, 2018).

2.8. USES OF CASSAVA LEAVES EXTRACTS

Cassava leaves (*Manihot esculenta* Crantz) widely used as vegetables in many regions. The aims of this study were to analyze the effect of boiled cassava leaves on total phenolics, flavonoids, and its antioxidant activity. Cassava leaves extraction

was done by maceration method to obtain the methanol extract and infusion method to get the water extract. Based on phytochemical test, cassava leaves contained alkaloids, flavonoids, phenolic, tannins, and saponins. The highest levels of total phenolics and flavonoids contained in the methanol extract of *simplicia*, 30.57 mg GAE/g and 881.33 mg RE/g, respectively. Cassava leaves extract had potential as an antioxidant with the highest inhibition of DPPH radicals produced by the methanol extract of *simplicia* with IC₅₀ values of 92.10 mg/L and followed by methanol extract of boiled leaves, water extract of *simplicia*, and water extract of boiled leaves with IC₅₀ 144.28, 155.76 and 170.71 mg/L, respectively. In conclusion, the process of boiling cassava leaves could reduce total phenolic, total flavonoids and antioxidant activity (Hasim *et al*, 2016).

2.9. IN-VITRO ANTI-OXIDANT STUDY

This study aimed at determining the In-vitro antioxidant capacity, characterizing phytochemical constituents, assessing toxic and functional properties of African yam bean (*Sphenostylis stenocarpa*) seed-enriched Cassava product (*Pupuru*) flour blends using standard methods. *Pupuru* flour blends were produced from spontaneously-fermented cassava tubers substituted with African yam bean (*Sphenostylis stenocarpa*) seed (AYBS) (5% (EP5), 10% (EP10) and 15% (EP15), before toasting, cooling, milling, sieving and packaging. A commercial sample (CP) with 100% cassava and another produced in this study were used as controls. *In-vitro* 2,2-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging abilities, total flavonoid content (TFC), ferric reducing antioxidant power (FRAP) and total phenol content (TPC) increased significantly ($P = .05$) as AYBS enrichment levels increased. All the phytochemicals except tannins increased significantly ($P = .05$) as levels of enrichment with AYBS increased. Tannins, phytates, saponins, alkaloids and oxalates were 1.46 -2.87 (mg/g); 0.85-1.40 mg/100 g; 4.18-13.27 mg/g; 24.89-29.05 mg/g and 1.71-3.23 mg/g, respectively. The toxic constituent revealed that all the samples contained significantly different ($P = .05$) cyanide ranging from 0.87-2.51 mg/kg which reduced as AYBS level of inclusion increased. The functional properties of the samples were significantly ($P = .05$) enhanced with AYBS enrichment. Utilization of AYBS to enrich *Pupuru* increased its *In-vitro* antioxidant capacity and

phytochemical constituents, reduced the toxic cyanide content and enhanced the functional properties, hence, its suitability as a nutraceutical to delay the ageing process and prevent cardiovascular diseases (Folayemi Janet Isaac-Bamgboye *et al*, 2020).

Plants are the vital source of pharmaceutically important compounds with less or no adverse side effects. The current study was conducted to catalog the commonly used indigenous and medicinal plants of Thailand based on their phenolic acid content and antioxidant activity. The herbs were collected from Chiang Mai province, Thailand. The plants were extracted with 70% ethanol. The total phenolic acid content and antioxidant activity were evaluated. The ethanolic extract of plant samples was prepared. About 94-97% of inhibition of free radical was detected in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay tested against the plant samples. The ethanolic extract of *Anaxagoras luzonensis* Gray., *Terminal asp.*, *T.belerica* Roxb, *Terminalia chebula* Retz., *Albizia procera* Benth., *Harrisonia perforata* Merr., and *P. emblica* Linn. Exhibited 97.87, 96.08, 92.26, 86.74, 86.08, 84.47, and 83.13% of superoxide radical inhibition, respectively. The results suggested that *T.belerica* Roxb. Possessed high TEAC ability and DPPH radical scavenging capacity and *A. luzonensis* Gray. Exhibited high superoxide scavenging activity, when compared to that of the other tested samples. The additional detailed study is desirable to understand the complexity and distribution of bioactive compounds present in the commonly used plant species of Thailand (Chaiyavat chaiyasut *et al*, 2017).

2.10. ANTI-BACTERIAL AND ANTI-DIABETIC ACTIVITY OF CASSAVA LEAVES

The antibacterial activity from ethanol extracts and the most active fraction of cassava leaves against clinical isolates of *Staphylococcus epidermidis* and *Propionibacterium acnes*. Research carried out by the experimental method involved determination of plants, extraction with maceration method, and fractionation with liquid-liquid extraction, antibacterial activity testing of extracts and fractions by agar diffusion method. MIC values of ethyl acetate fraction against *S.epidermidis* were in the concentration range of 2.5%–5.0% (w/v) and against *P.acnes* were in the concentration range of 1.25%–2.5% (w/v). (e MBC value of ethyl acetate fraction against *S.epidermidis* was at a concentration of 5% (w/v), while *P.acnes* was at a

concentration of 2.5% (w/v). From the results of this study, it can be concluded that the ethanol extract of cassava leaves (*Manihot esculenta Crantz*) has antibacterial activity against clinical isolates of *Staphylococcus epidermidis* as well as on *Propionibacterium acnes*. (Fraction with the best activity from the ethanol extract of cassava leaves to the two test bacteria was shown by ethyl acetate fraction. It is suggested that cassava leaves are possible to be developed into standardized anti acne herbs (Resmi Mustarichie *et al*, 2020).

Kasoami, traditional food from Buton, is a functional food with antidiabetic activity. Kasoami is made up of Kaopi from cassava (*Manihot esculenta Crantz*). This study aimed to examine the Kaopi and Kasoami from white cassava as antidiabetic food by its antioxidant capacity. Kaopi and Kasoami were macerated with ethanol. The extracts obtained were qualitatively screening, and quantitatively measured their total phenolic and flavonoid content by colorimetric methods. Simultaneously, Kaopi and Kasoami were assayed their antioxidant capacity by DPPH and ABTS methods, and the antidiabetic was assayed in vivo. The phenolic compounds and flavonoids in Kaopi and Kasoami from white cassava provided antioxidant capacity, thus decreased blood glucose levels in diabetic-induced mice models (Haidir Amin *et al*, 2020).

2.11. COMPARATIVE STUDY ON WILD AND MICRO PROPAGATED CASSAVA ETHANOL EXTRACT

A comparative study of the phytochemical constitutes antioxidant and cytotoxicity activities of wild and micro propagated *Manihot esculenta* ethanol (WMEE and MMEE) extracts. In micro propagation of *M.esculenta*, the plant growth regulators combination 6-benzyl amino purine (8.88 μ M) along with naphthalene acetic acid (0.98 μ M) and 1.16 μ M kinetin (1.16 μ M) were observed to have the best shoot formation. Wild and micro propagated plants were extracted by ethanol which extracts the phytochemical quantitative screenings, FTIR and GCMS for identification of phytocompounds. The major secondary metabolites were presented in both sample extracts which are confirmed by preliminary phytochemical quantification tests. WMEE and MMEE extracts phytochemical constitutes were identified by FTIR and GC-MS analysis. In FTIR spectrum results, five and two major

peaks were reported and 80 to 85 phytochemicals were identified by GCMS. Further, both sample extracts were studied for the antioxidant property by various methods and colon carcinoma cell viability through MTT assay. In antioxidant activity, the WMEE and MMEE extracts showed significant activity in hydrogen peroxide scavenging. The cytotoxicity result of the WMEE extract was more potent in colon carcinoma cells than MMEE extract. From the phytochemical and pharmacological studies, the WMEE and MMEE extracts reported almost similar biological activities. So, the micro propagated plants will be used instead of wild plants for pharmaceutical purposes (Vajjiram Chinnadurai *et al*, 2019).

2.12. EVALUATION OF TWO MONTH OLD FOURTEEN BREEDING LINES OF *MANIHOT ESCULENTA*

Two (2) month-old leaves of fourteen (14) breeding lines of cassava, consisting of five (5) parental lines and nine hybrids, were evaluated for their phytochemical constituents. The objective of the study was to determine total flavonoid, phenolic and antioxidant activity in the leaves of the breeding lines. The 14 breeding lines were grown in the research farm of the Biotechnology and nuclear Agriculture Research Institute between March and September 2011. Analyses were carried out at the laboratories Ghana Atomic Energy Commission (GAEC) between July and August 2011. The randomized complete block design, with three replicates, was used. Results indicate statistically significant differences in Total Flavonoid Contents (TFCs), Total Phenolic Contents (TPCs) and Total Antioxidant Activities (TAAs) recorded for both the ethanolic and aqueous extracts of the breeding lines. Hyb-9 gave the highest total flavonoid content of 179.90 ± 0.21 mg/g/QE (ethanol extract) as well as total phenolic contents of 128.25 mg/g/GAE and 95.33 ± 3.61 mg/g/GAE for both ethanol and aqueous extracts, respectively, while Security gave the highest value for total flavonoid content of 96.7 ± 0.03 (aqueous extract). Similarly, Larbire corded the highest TAA in ethanolic extract ($82.88 \pm 3.07\%$), while Hyb-15 gave the highest value of aqueous extract ($80.92 \pm 2.79\%$). In general there was strong positive correlation among the TFC, TPC and TAA. Most hybrids exhibited higher TFCs and TPCs than their parents in the ethanolic extracts (Quartey *et al*, 2016).

2.13. CASSAVA TUBER IN FOOD PACKAGING PRODUCTION

Yams and other tubers such as potatoes, cassava and sweet potatoes represent crops of great economic importance worldwide, constituting the main source of income and employment in many rural areas of the world. The present investigation proposes the use of native starches as raw material in the production of food packaging, which were modified chemically by reaction of hydrolysis and esterification (DDSA, OSA and AA). Starches and films based on native and modified starches were characterized by FTIR spectroscopy and the results revealed that employed reactions can add hydrophobic functional groups to improve the water resistance of the biodegradable films based on starches (Torrenegra *et al*, 2018).

2.14. NUTRITIONAL AND OTHER ACTIVITY OF *IPOMOEA BATATAS* PEELS AND *MANIHOT ESCULENTA* PERIDERM AND CORTEX LAYERS

This study is reported the nutritional composition, phytochemicals and antioxidant activity of red and purple variety of *Ipomoea batatas*(sweet potato) peels and *Manihot esculenta*(cassava) periderm and cortex layers. The proximate composition (carbohydrate, protein, fat, moisture, ash and minerals) was determined using standard methods while phytochemical contents (phenolic, flavonoid, anthocyanin and carotenoid) and antioxidant activity [2,2-diphenylpicrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays] were determined spectrophotometrically. Proximate analysis showed that both *I.batatas* and *M.esculenta* contained high carbohydrate content ranging from 75.07% to 87.04% but zero protein and low-fat contents (0.83-2.2%). For the antioxidant study, both purple and red varieties of *I.batatas* showed higher percentages of inhibition using DPPH assay with 87.93% and 87.62%, respectively compared to *M. esculenta*. Meanwhile, FRAP assay displayed lower values than ABTS assay for both tubers. In conclusion, both tubers, *I.batatas* and *M.esculenta* have close values of nutritional compositions. However, *I.batatas* showed higher mineral contents (potassium, iron, magnesium and sodium) and antioxidant activity than *M.esculenta*. For the phytochemical content, *I.batatas* contained higher total flavonoid and total anthocyanin contents while *M.esculenta* contained higher total phenolic and carotenoid contents. Hence, this study

suggested that tuber's by-products have great potential for use in functional food as they are cheap and readily available as well as reducing waste disposal (Dusuki *et al*, 2019).

2.15. AROMATIC COMPOUND EXTRACTION OF CASSAVA FLAKES

Cassava flakes and “garri” are some of the popular cassava products consumed in Africa, Southeast Asia, and Brazil. These products are cherished for their creamy colour and characteristic aroma. The aroma compounds responsible for the unique aroma notes of these products were evaluated by means of solid-phase micro-extraction (SPME). Results confirmed that the divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) fibre was the most appropriate for the isolation of aroma compounds in the cassava products. In addition, the best response was attained when the extraction temperature was 60°C, equilibrium time 20 min, extraction time 10.94 min, and water addition 35%. Analysis of the odorants led to the identification of 21 compounds with an array of odour notes. However, results of the aroma extract dilution analysis (AEDA), and odour activity values (OAVs) showed that guaiacol, 3-methylbutanal, methylpropanal, and butyric acids contributed intensely to the characteristic aroma of both cassava flakes and garri (Ola Lasekan *et al*, 2016).

2.16. CHEMOTYPES OF CASSAVA BREEDING PROGRAM

Cassava (*Manihot esculenta* Crantz) is the predominant staple food in Sub-Saharan Africa (SSA) and an industrial crop in Southeast Asia. Despite focused breeding efforts for increased yield, resistance, and nutritional value, cassava breeding has not advanced at the same rapidity as other staple crops. In the present study, metabolomics techniques were implemented to characterize the chemotypes of selected cassava accessions and assess potential resources for the breeding program. The metabolite data analyzed was applied to describe the biochemical diversity available in the panel, identifying South American accessions as the most diverse. Genotypes with distinct phenotypic traits showed a representative metabolite profile and could be clearly identified, even if the phenotypic trait was a root characteristic, e.g., high amylose content (Margit Drapal *et al*, 2019).

2.17. STUDY OF DIFFERENT ROOT CROPS AGAINST PATHOGENIC FUNGI

The *in vitro* efficacy of aqueous leaf extracts of three common root crops (*Colocasia esculenta*, *Manihot esculenta* and *Dioscorea rotundata*) were investigated against *Aspergillus niger* and *Botryodiplodia theobromae*. The pathogenic fungi were isolated from rotten tubers of yam using antibiotic-amended potatoes dextrose agar. Though, *M.esculenta* leaf extracts generally demonstrated the least antifungal activities against both pathogens, their activities were significant at 75 g/L. The antifungal potency of both *D.rotundata* and *C.esculenta* were comparable to Camabez which demonstrated 100 %inhibition at both 5 and 10 g/L concentrations. Higher concentrations of saponins (5.64 -6.71 mg/100g), tannins (4.42 – 7.23 mg/100g) and flavonoids (3.64 – 5.25 mg/100g) were detected in the extracts, and could probably be linked to the observed antifungal activities. Findings from this study suggest that *D.esculenta*, *C.esculenta* and *M.esculenta* leaf extracts possess bioactive molecules that could be exploited in the control of phytopathogens of tubers crops, which are the main staple food in most tropical countries (John and Gideon, 2018).

2.18. EFFECT OF POLYPHENOLS IN CASSAVA LEAVES

The study was carried out to assess the effect of variety on polyphenols in cassava leaves and their stability in antioxidant activity before and after *in vitro* gastrointestinal digestion. The results showed that individual and total polyphenols content (TPC) and antioxidant activity of bound, free and bioaccessible polyphenols were significantly ($p < 0.05$) influenced by variety at harvesting maturity. The free polyphenols of variety IRAD4115 had the highest value of FRAP (35.17 $\mu\text{g TE/g}$) at 12 months after planting (MAP), while, bound polyphenols showed the lowest DPPH (6.59 $\mu\text{g TE/g}$, variety EN at 12MAP). The antioxidant activity value evaluated by DPPH method was decreased significantly after *in vitro* gastrointestinal digestion. However, there was no significant difference between antioxidant activity of bioaccessible polyphenols (77.71 $\mu\text{g TE/g}$) and methanolic polyphenols (79.17 $\mu\text{g TE/g}$) assessed by FRAP (Ferric reducing ability of plasma) method. These findings showed the stability of antioxidant potential of polyphenols in cassava leaves

harvested at different periods after in vitro digestion. Thus cassava leaves harvested at appropriate maturity can be used as an ingredient of functional food for nutraceutical benefits (Alphonse Laya and Benoît, 2020).

2.19. DIFFERENT SPECIES OF PLANT POWDER AGAINST *ORYZAEPHILUS SURINAMENSIS*

This study was carried out to investigate whether powdered plants of different species namely: peppermint *Mentha piperita* (L.) (Lamiaceae), wormwood *Artemisia absinthium*(L.) (Asteraceae), common sage *Salvia officinalis*(L.) (Lamiaceae), allspice *Pimenta dioica* (Linnaeus et Merrill) (Myrtaceae) and common garlic *Allium sativum* (L.) (Amaryllidaceae), added to semolina using concentrations of 1.23, 3.61, and 5.88%, influence the mortality rate in the saw-toothed grain beetle *Oryzaephilus surinamensis* Linnaeus, 1758(Coleoptera: Silvanidae). Experiments were conducted in a laboratory at 28°C and relative humidity 60±5%. At the concentration of 1.23%, allspice seeds caused the highest mortality amongst the saw-toothed grain beetle. When concentrations of 3.61 and 5.88% were used, sage, peppermint and wormwood caused the highest statistically significant mortality of *O.surinamensis* (Małgorzata Kłys and Anna Przystupinska, 2015).

2.20. MORPHO AGRONOMIC AND INDUSTRIAL PERFORMANCES OF THREE CASSAVA CLONES

This research work was done to study the morpho agronomic and industrial performance of three cassava clones. The study was carried out in two stages: A) the MMEXV5, MMEXV40, and MMEXCH23 clones were established in a sub humid warm climate; B) the storage roots were processed. For the first essay, a randomized complete block design was used, while for the second essay, a completely randomized design was used. An ANOVA analysis was done, as well as a means comparison (Tukey, 0.05) and a Principal Component Analysis (PCA). Additionally, the three clones reached high dry matter content (DM = 34.22 to 38%), trait considered a quality factor. The PCA showed that RY was associated with a higher number of storage roots generated, PH, PUL, and lobe dimensions; but, the

clones with high RY and PH developed poor DM and yield of starch extraction. Finally, the valorization of the evaluated germplasm could make cassava into the basic raw material in a great variety of products with high added value for the food and non-food industry; even obtain bioproducts and bioenergy through the conversion of bagasse and peel (Jose Luis Del Rosario-Arellano *et al*, 2020).

2.21. ANTAGONISTIC PROPERTY AND FERMENTATION OF CASSAVA STARCH

The antagonistic properties of indigenous micro flora from cassava starch, flour and grated cassava were investigated using the conventional streak, novel ring and well diffusion methods. Antagonism was measured by the zone of inhibition between the fungal plug and bacterial streak/ring. *Bacillus* species were more effective to inhibit the growth of *Aspergillus niger*, *A.fumigatus*, *Fusarium moniliforme* and *Rhizactonia* sp. *Pseudomonas fluorescens*, *Escherichia coli* and *Saccharomyces* sp. inhibited the growth of *A.niger*, *A.flavus* and *F.moniliforme*. There were considerable variations in inhibitory activity. The zone of inhibition was more apparent in the novel ring method. Percentage inhibition increased to 78% in the Ring method and 62% in streak method after 168 h of incubation. *Lactobacillus brevis*, *L.acidophilus* and *Bacillus subtilis* inhibited the growth of *E.coli* and *Staphylococcus aureus*. Inhibition of the pathogenic microorganisms was probably due to the production of organic acids and bacteriocins. (Agarry *et al*, 2020).

Sour cassava starch is a product obtained from cassava (*Manihot esculenta* Crantz) processing to defeat raw material toxicity and low nutritional value (lipids and proteins). The aim of this work was to produce sour cassava starch in a pilot-scale fermentation process with *Lactobacillus plantarum* a single culture and in co-culture with *Pichia scutulata* and to determine the fatty acids and volatile compounds profile. The products obtained with single and mixed cultures exhibited a reduction of saturated fat (3.6 % and 8.1 %, respectively), an increase of the unsaturated fat (2.3 times), and an increase of the oleic, linoleic and linolenic acids proportion. These fatty acids have a positive effect on human health and well being. A greater variety of desirable volatile compounds was a differential of the product obtained with the mixed culture. Therefore, *L.plantarum* with *P.scutulata* was the starter culture that

exhibited better results in the pilot-scale fermentation process and has the potential for the industrial production of sour cassava starch (Fernanda *et al*, 2019).

2.22. ANTI-FUNGAL ACTIVITY FROM THE EXTRACT OF CASSAVA ROOTS

The chemical contamination of food is among the main public health issues in developing countries. With a view to find new natural bioactive products against fungi responsible for chemical contamination of staple food such as maize, the antifungal activity tests of scopoletin extracted from different components of the cassava root produced in Benin were carried out. The dosage of scopoletin from parts of the root (first skin, second skin, whole root, and flesh) was done by High Performance Liquid Chromatography. The scopoletin extract was used to assess the activity of 12 strains (11 strains of maize and a reference strain). Scopoletin extract from the cassava root skins showed significant inhibitory activity on the tested strains with fungicide concentration (MFC) between 0.0125mg/mL and 0.1mg/mL. The antifungal scopoletin extracted from the cassava root skins may be well beneficial for the fungal control of the storage of maize (Rafiatou Ba *et al*, 2017).

2.23. REPELLENT BHAVIOURAL STUDY AGAINST *ORYZAEPHILUS SURINAMENSIS*

Saw-toothed grain beetle, flat grain beetle and red flour beetle are major among the stored grain insect population. These insects destroy at least 15% of the food produced in India as they feed almost entirely on the germ of the grain reducing its quality. For selection of control measure advantages, disadvantages and safety towards grain quality must be the first consideration. Plants are composed of many constituents some of them are insecticidal, attractants, repellent, antifeedants, hormone mimics and hormone antagonists. Repellent behavior is one of the important measures as it does not kill the insect but protect the grain from infestation. Here some plant extract *Punica granatum*, *Chenopodium album*, *Vitex negundo* and *Maytenus emarginata* and their combinations were applied to test insect *Oryzaephilus surinamensis* at the dose of 1, 2.5 and 5 ml /100 gms of cashew nuts to control them through repellent behavior (Neetu Kachhwaha *et al*, 2015).

2.24. EFFECTS OF *MANIHOT ESCULENT* ON IMMUNE SYSTEM OF *SPODOPTERA LITURA*

Nowadays, the use of chemical insecticides has led to development of resistance in insects, and causing harmful effects to non - target organisms and environment. Plant - mediated biopesticides are an alternative source for the management of insect pests. Present study focuses on lethal and sub lethal effects of *Manihot esculenta* on immune response and antioxidant enzyme activity of *Spodoptera litura* larvae. The result shows the high toxicity of petroleum benzene leaf extract in *S. litura* larvae with LC 50 of 109.89 mg/ mL. FT - IR analysis of leaf extracts shows the presence of alkane, alkyl halides, misc, amines, esters and carboxylic acid. GC - MS analysis of extracts contains 10 compounds, notable among them are Octadecadienoic acid, Lupeol and Octamethyl. There was an increase in the total haemocyte count and differential haemocyte count in *S.litura* larvae. Phenoloxidase (PO) levels, detoxification enzymes such as Cyt P450, GST and catalase levels were significantly increased, whereas, SOD levels were lower than the non - treated *S. litura* larvae. *Manihot esculenta* leaf extracts is an alternative to chemical insecticides for the management of *S.litura* larvae and may be used for the management of other agricultural insect pests (Palanisamy Manjula *et al*, 2020).

2.25. FORMULATION OF BIOPESTICIDES WITH CASSAVA LEAF EXTRACT

Present investigation was carried out to formulate an effective bio pesticide formulation against two major sucking pests of field crops. Neem oil, surfactant and cassava leaf extract were the three constituents used in the formulations. Major field pests, the papaya mealy bug, *Paracoccus marginatus* and cowpea aphid, *Aphis craccivora* were selected for the study. A total of six formulations at concentration of 2, 1 and 0.5 were sprayed on mealy bug infested papaya seedlings and aphid infested cowpea plants. The mortality percentage varied according to formulations and 1% formulation F which contained 50 ml neem oil, 30 ml surfactant and 20 ml cassava leaf extract proved to be the most effective bio pesticide formulation against these sucking pests. Accordingly, the amount of neem oil required for control of these pests was reduced due to the additive action of other constituents (Jayaprakas and Sreerag, 2015).

CHAPTER 3

AIM AND SCOPE

3.1. AIM

This study is aimed to investigate the biopesticidal activity of *Manihot esculenta* against *Oryzaephilus surinamensis* Linn. (Storage pest).

3.2. SCOPE

The main scope of the study is to highlight the biopesticidal effect which is derived from *Manihot esculenta* (cassava leaves) against *Oryzaephilus surinamensis* Linn. This study stresses on the adverse effects caused by the chemical pesticides and to prove that Biopesticides from *Manihot esculenta* provide a much safer and effective alternative source for synthetic pesticides against the pest. The crude extracts of the cassava leaves are obtained using three different solvents such as methanol, ethyl acetate and chloroform these crude extracts of the plant are tested against *Oryzaephilus surinamensis* (L.). The molecular docking study is performed for those compounds eluted by GC-MS analysis.

3.3. PLAN OF WORK

- Collection of plant material
- Crude extraction of plant material
- Preliminary screening of crude extract
- Pesticidal effect against *Oryzaephilus surinamensis*.
- Purification of crude extract
- GC-MS analysis
- Molecular docking

CHAPTER 4

MATERIALS AND METHODS

4.1. COLLECTION OF PLANT MATERIALS

The fresh leaves of *Manihot esculenta* (cassava) were collected from the field located in Chengalpattu, Tamil Nadu, and India. 500 g of cassava leaves were collected and washed using distilled water and placed in a filter paper and air dried under shade for ten days until it becomes brittle and crunchy.

4.2. PREPARATION OF PLANT EXTRACT

The dried leaves are grinded and weighed. 65 g of powdered leaves sample is obtained from 500 g of dried leaves. 20 g of leaves powder were taken in a three different clean and dry conical flask. The extraction was performed by maceration method. The three different solvent systems which are used for extraction are methanol, ethyl acetate and chloroform, 20 ml of each solvent were taken in a respective conical flask containing leaves powder and left in a shaker for 48 hours. After 48 hours the filtrate is collected using whatman filter paper, this process is performed three times and they were filtered. The filtered solvents were then poured in three different glass bowls for condensation for three days. After which 2 g, 1.48 g, and 1.67 g of crude were obtained from methanol, ethyl acetate, and chloroform extracts respectively. The crude is stored in room temperature for further analysis.

4.3. PRELIMINARY SCREENING OF CRUDE EXTRACTS

4.3.1. Phytochemical analysis

The crude extracts were screened to identify the secondary metabolites such as tannins, alkaloids, saponins, glycosides, terpenoids, flavonoids etc. and active substances found in the plants.

4.3.1.1. Test for Alkaloids (Harborne, 1980)

For preparation of stock solution, 3 ml of extract was added with 3 ml of 1% diluted HCl and kept in water bath for 5 mins. To 1ml of stock solution, few drops of Mayer's reagent was added and checked for creamy white precipitate.

4.3.1.2. Test for Terpenoids (Salkowski test)

2 ml of chloroform was added to 100 mg of crude extract and mixed well. Then 1ml of concentrated sulphuric acid was added slowly along the sides of the test tube. Formation of reddish brown ring confirmed the presence of terpenoids in the crude extract.

4.3.1.3. Test for Tannins (Lead acetate test)

To 1ml of extract, few drops of 5% lead acetate was added and shaken well. Formation of white colour precipitate confirmed the presence of tannins.

4.3.1.4. Test for saponins (Kokate, 1999).

To 2ml of extract, 2 ml of distilled water was added followed by vigorous shaking. The mixture was warmed until stable foam was formed.

4.3.1.5. Test for Flavonoids

To 1ml of extract, few drops of 2% sodium hydroxide solution was added and checked for formation of yellow precipitate.

4.3.1.6. Test for Steroids (Liebermann-Burchard's test)

To 1ml of extract, 1 ml of acetic anhydride was added and shaken well. To this, few drops of concentrated sulphuric acid were added along the sides of test tube. Appearance of dark green colour confirmed the presence of steroids.

4.3.1.7. Test for Glycosides (Keller killani test)

1ml of crude extract is shaken with 1ml of distilled water followed by addition of 1ml of glacial acetic acid. Few drops of ferric chloride is also added along with 1ml of sulphuric acid after which formation of brown ring confirmed the presence of glycosides.

4.3.1.8. Test for Amino acids (ninhydrin test)

To 2ml of extract, few drops of 2% ninhydrin solution were added. Then test tube must be kept in warm water bath for 5 mins. Formation of purple colour indicates the presence of amino acids.

4.3.1.9. Test for carbohydrate (Molish Test)

To 2 ml of extract add few drops of alpha-naphthalene and shaken well. Formation of violet ring confirmed the presence of carbohydrates.

4. 3.1.10. Test for Phenolic compounds (FeCl₃ test) (Mace, 1963)

The extract (20 mg) was dissolved in 2 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A deep dark green colour indicated the presence of Phenolic resin compounds.

4.3.2. ANTIMICROBIAL ACTIVITY

4.3.2.1. ANTIBACTERIAL ACTIVITY

The microorganisms of gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Pseudomonas aeruginosa* were used for the evaluation of the antibacterial activity. Antibacterial activity of the crude extract of *Manihot esculenta* was carried out using agar well diffusion method (Eloff, 1998).

Agar Well Diffusion Method

Nutrient agar medium was prepared according to the standard methods (peptone-5 g, yeast extract-3 g, NaCl-5 g, distilled water- 1000ml, agar- 20 g). The required amount of ingredients was weighted and suspended in the required volume of 100ml of distilled water and then autoclaved at 15 lbs and 121°C for 15 minutes.

The hot medium was poured in sterile petri plates which were kept in the aseptic laminar chamber. The medium was allowed to solidify for 15 min. The solidified nutrient agar in the petri plates was inoculated by dispensing the inoculums using sterilized cotton swabs which are previously immersed in the inoculums containing test tube and spread evenly all the sides onto the solidified agar medium. Five wells

were created in each plate with the help of a sterile well-borer of 6mm diameter. The methanol crude extract, ethyl acetate crude extract and chloroform crude extract of *Manihot esculenta* was then poured into each well containing 250 µg/mL concentration. All the plates with extract loaded wells were incubated at 37°C for 24 h and the antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well. Tetracycline (25 µg) was used as the positive control.

4.3.2.2. ANTIFUNGAL ACTIVITY

The screening of crude extract for their antifungal activity against the given test fungal pathogen. The microorganism such as *Fusarium solani* and *Rhizactonia solani* were used for the evaluation of the antifungal activity. Antifungal activity of the crude extract of *Manihot esculenta* was carried out using agar well diffusion method.

Agar Well Diffusion Method

Potato dextrose agar medium was prepared according to the standard methods (potato peeled – 200 g, dextrose – 20 g, agar- 20 g, distilled water – 1000 mL). The pH range was between 7.0 – 7.5. Molten PDA was poured into a sterilized petri dish, which were kept in the aseptic laminar chamber. The medium was allowed to solidify for 15 min. The solidifies PDA in the petri plates was inoculated by dispensing the inoculums using sterilized cotton swabs which are previously immersed in the spores containing test tube and spread evenly all the sides onto the solidified agar medium. Four wells were made on agar plate with the help of a sterile well-borer of 6mm diameter. The 250 µg/mL concentrations of all the three solvent crude extract of the plant were loaded into the wells. Fluconazole was used as positive control. The plates were then incubated at 37°C for 48 hours. After incubation diameter was measured using zone scale.

4.4. PESTICIDAL ACTIVITY AGAINST ORYZAEPHILUS SURINAMENSIS

The pest *Oryzaephilus surinamensis* were got from the stored rice (*Oryza sativa*) from University of Madras, 9/36, Gandhi Mandapam road, Anna University, Kotturpuram, chennai, Tamil Nadu 600085 was collected and stored at room temperature and fed with fresh and untreated wheat grains. The pesticidal activity was carried by contact toxicity method in university of madras, glass vials were used

for this experiment, the internal dimensions of the vials 8 x 1.1 cm (height, radius), was taken for screening of pesticidal activity. Five vials were taken for each sample and they were labeled with different concentration respectively for each sample. 1ml aliquot of each extract with different concentration (62.5, 125, 250, 500 and 1000 ppm) was uniformly applied to the inner wall of each vials and were air- dried for 5 min this step is repeated for five times before introduction of adult for complete evaporation and drying of the acetone. Control vial was applied only with acetone. Ten adults were introduced into treated vials followed by adding 1g of feed (Broken wheat), for each concentration five replicates were used. The process was repeated for all the extracts. The Adult mortality was assessed after 24 hours and 48 hours of treatment. Percentage insect mortality was calculated using the Abbott's correction formula (Abbott, 1925). The obtained data were subjected to statistical analysis – ANOVA to prove the significance of the results (Fatima et al, 2016).

$$\text{Abbott value} = \frac{\text{Percentage of treated} - \text{percentage of control}}{100 - \text{Percentage of control}} \times 100$$

Where percentage of treated = Treated / total no of adult treated x 100

Percentage of control = control / total no of adult control x 100

4.5. ANTI-OXIDANT ASSAY- DPPH Free radical scavenging activity

Anti-oxidant is a substance that prevents destruction by oxidation. Oxidation is an important process in all living organisms in order to fuel the biological processes that occur within them.

A free radical is a compound with one or more unpaired electron in its outer orbital, these unpaired electrons are quite reactive with other molecules which make these species very unstable. Similarly, ROS- a reactive oxygen species a not technically a free radical are also highly reactive with other compounds around them which will lead to many diseases, and cell death. Therefore, the antioxidant activity of methanol extract of *Manihot esculenta* was evaluated through free radical scavenging effects

on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The free radical scavenging activity of the crude extract was evaluated in terms of % radical scavenging activity. Gallic acid was taken as standard to compare them with the values of the methanol crude extract. Different concentration of methanol plant extract such as 20, 40, 60, 80 and 100 µg/ml were taken. The determination was based on the method proposed by (Akowuah et al., 2005). 1 mL of 0.1mM DPPH methanolic solution was added into 1mL of sample extracts. The mixture was thoroughly mixed and kept in the dark for 30 minutes under room temperature. The colour change was observed after incubation time after which the absorbance was read at 515nm (Bhuiyan et al., 2009) against a reagent blank. The radical scavenging activity was calculated from the formulae.

$$\% \text{ inhibition of DPPH} = [\text{Control} - \text{Sample}/\text{Control}] \times 100s$$

The graph was plotted against the concentration of the sample to determine the IC₅₀ value of the crude extract. IC₅₀ value is the concentration of the sample required to scavenging 50% of the DPPH free radicals. Studies show that lower the IC₅₀ value, higher is the antioxidant activity.

4.6. PURIFICATION OF CRUDE EXTRACTS

The purification of crude extracts was performed through chromatography techniques – Thin layer chromatography.

4.6.1. Thin layer chromatography

Thin-layer chromatography is a technique used to isolate the non-volatile mixtures. TLC depends on the separation principle.

The separation relies on relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that compounds which has higher affinity to stationary phase move slowly while other compounds travel fast. Thin-layer chromatography was done as a preliminary method in order to detect the solvent system for separation of maximum number of compounds in column chromatography.

Thin layer chromatography was carried out for all the three extracts of *Manihot esculenta* in Merck TLC aluminium sheet, silica gel 60 F254 (20 x 20cm), preloaded plate. The extract was spotted by using capillary tubes at 0.3 mm above from the bottom of the TLC plate. The chromatogram was developed in a mixture of suitable solvent system. The developed TLC plates were air dried and visualized for presence of bands (Kagan & Flythe, 2014). R_f (retention factor) were calculated from the below formula for each band developed,

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

4.7. FOURIER- TRANSFORM INFRARED SPECTROSCOPY

Fourier- transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. FTIR (Fourier Transform Infrared) spectrometer obtains an infrared spectrum by first collecting an interferogram of a sample signal using an interferometer, then performs a Fourier Transform on the interferogram to obtain the spectrum. It is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular “fingerprint”. The wavelength of the light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. The FTIR spectrometer uses a Michelson interferometer. The functional groups present in the crude extract were identified using FTIR.

4.8. GAS CHROMATOGRAPHY – MASS SPECTROSCOPY

GC-MS is the analysis method of choice for smaller and volatile molecules such as benzenes, alcohols and aromatics, and simple molecules such as steroids, fatty acids, and hormones. It can also be applied towards the study of liquid, gaseous and solid samples. A Shimadzu GC- 20210 plus gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and 15m Alltech EC-5 column and a direct connection with capillary column metal quadupole mass filter per rod mass spectrometer operating in electron ionization (EI) mode with software GCMS solution ver. 2.6 was used for all analyses. GC-MS was done in order to identify the different number of components of the compound was done by matching their recorded spectra with the data bank mass spectra of NIST library V11 provided by the

instrument's software. GC-MS metabolomics database was used for the similarity search with retention index.

4.9. MOLECULAR DOCKING

Molecular docking is defined as an optimization problem, which would describe the best-fit orientation of a ligand that binds to a particular receptor of interest and is used to predict the structure of the intermolecular complex formed between two or more molecules. It provides useful information about drug receptor interactions and is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of small molecule. Molecular visualization is a key aspect of analysis and communication of modeling studies. It enables a mechanistic understanding of a molecule's structure to be visualized, so that key insights can be shared between computational modeling experts and collaborating team members (Sharma & Singh, 2014). The process starts with crystal coordinates with target receptor, generates molecular surface for receptors, also generates spheres to fill the active site of the receptor where sphere becomes potential locations for ligand atoms, sphere centers are then matched with the ligand atoms, to determine possible orientations for the ligand and finally find the top scoring or best ranking.

The ligands present in the methanolic extract of the plant was chosen based on the review of literature, which are oleic acid, elaidic acid isopropyl ester, 14-methyl-18-oxononadec-14-enoic acid methyl ester and flavone (which is responsible for the pesticidal activity) was taken from protein data bank along with the receptor present in the gut region of the pest. And the docking was done in discovery studio 2020 part of the 2020 BIOVIA product release series. And scoring was obtained by comparing the sites of interactions between ligands and receptors.

CHAPTER 5

RESULT AND DISCUSSION

5.1 PREPARATION OF CRUDE EXTRACT

The methanol, ethyl acetate and chloroform extract of *Manihot esculenta* was obtained as a result of double extraction and 60 ml each of total solvent were extracted. After condensation, the crude was obtained and weighed;

Methanol : 2 g

Ethyl acetate: 1.48 g

Chloroform : 1.67 g



Fig: 5.1: Solvent extraction

5.2 PRELIMINARY SCREENING OF CRUDE EXTRACTS

5.2.1 PHYTOCHEMICAL ANALYSIS

From the results, it was found that alkaloid was present in all extracts. Literature study reveals that the presence of alkaloids in plants indicates the biopesticidal activity. Flavanoids was present in methanol and ethyl acetate extracts. Tannins are

present only in ethyl acetate extract. Terpenoids was present in all extracts. The results of phytochemical analysis of three solvent extracts is summarized in the below (Table 5.1).

Table 5.1 Phytochemical analysis

NAME OF THE TESTS	METHANOL	ETHYL ACETATE	CHLOROFORM
ALKALOIDS Mayer's test	+	+	-
ALKALOIDS Wagner's test	+	+	+
TERPENOIDS Salkowski test	+	+	+
STEROIDS Libermann's test	+	-	+
FLAVANOIDS Alkaline reagent	+	+	-
CARBOHYDRATE Molish test	+	-	+
GLYCOSIDES Keller kellani's test	+	+	+
TANNINS Lead acetate test	-	+	-
AMINO ACID Ninhydrin	+	-	-
SAPONINS	+	-	-
PHENOLIC COMPOUNDS Ferric chloride test	+	+	+



Fig: 5.2: phytochemical analysis for methanol extract of Manihot esculenta



Fig: 5.3: phytochemical analysis for ethyl acetate extract of Manihot esculenta



Fig: 5.4: phytochemical analysis for chloroform extract of *Manihot esculenta*

5.2.2 MICROBIAL ACTIVITY

5.2.2.1 Antibacterial activity

The antibacterial activity of methanol, ethyl acetate and chloroform extract of *Manihot esculenta* were studied against four Gram-negative pathogenic bacterial strains (*E.coli*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Pseudomonas aeruginosa*). No zone of inhibition was found (Fig 5.5). No activity was found which indicates that the plant *Manihot esculenta* (cassava leaves) does not have antibacterial activity. Therefore, this plant is more prone to plant pathogens.

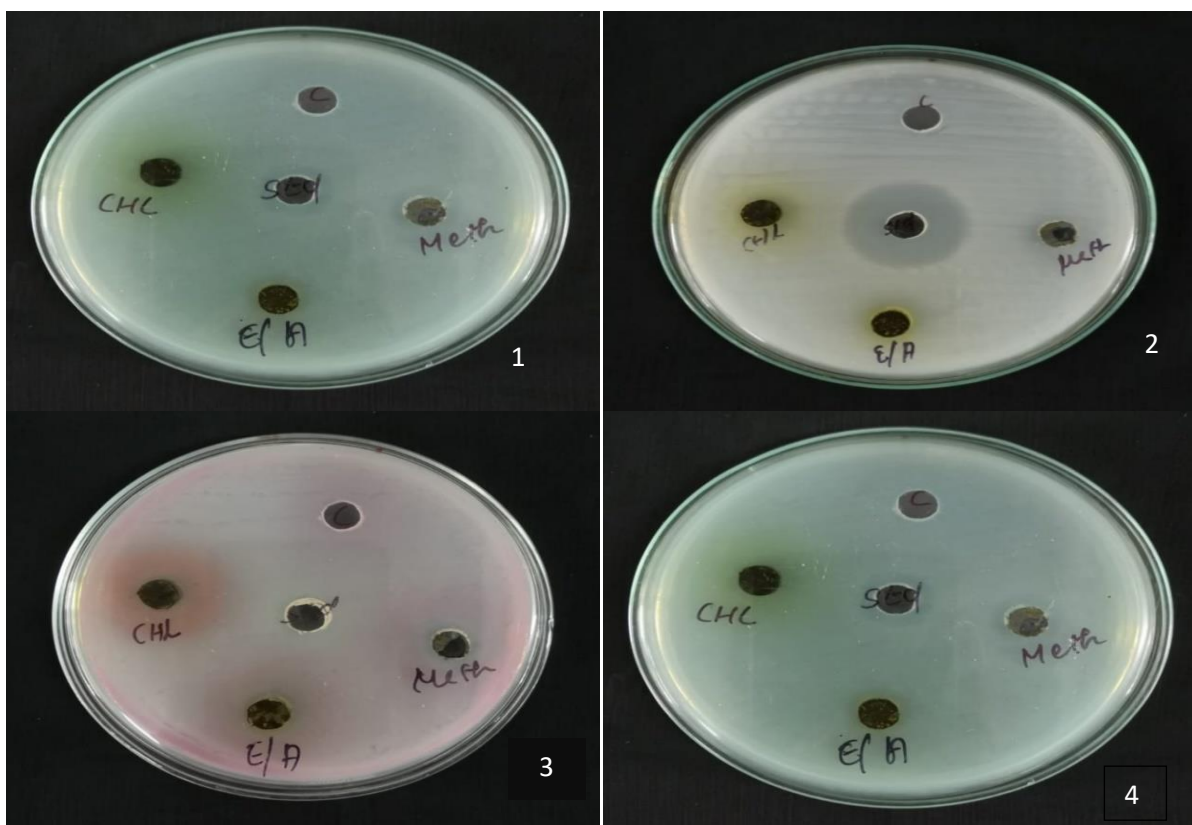


Fig: 5.5: Anti-bacterial test result (1. *Escherichia coli*, 2. *Serratia marcescens*, 3. *Klebsiella pneumoniae*, 4. *Pseudomonas aeruginosa*).

5.2.2.2 Antifungal activity

The antifungal activity of methanol, ethyl acetate and chloroform extract of *Manihot esculenta* against two fungal strains (*Fusarium solani*, *Rhizactonia solani*). No zone of inhibition was found (Fig: 5.6). The crude extract of the plant cassava leaves does not possess any anti- fungal activity against plant pathogens.



Fig: 5.6: Anti-fungal test result (1. *Fusarium solani*)



Fig: 5.7: Anti-fungal test result (2. *Rhizactonia solani*).

5.3. PESTICIDAL ACTIVITY AGAINST *Oryzaephilus surinamensis* (STORED PEST)

The three solvent extract of *Manihot esculenta* were tested against the stored pest *Oryzaephilus surinamensis* for the period of 48 hours. The mortality rate of the pest was observed at 24hr and 48hr of interval. The death of the *Oryzaephilus surinamensis* was confirmed when no leg or antennal movements were observed after 48 hours of exposure. The mortality rate was calculated for all the three crude extract – methanolic extract, chloroform extract and ethyl acetate extract. The highest mortality rate was found in methanolic crude extract of *Manihot esculenta*. This may be due to the presence of flavonoids present in the methanolic extract which are reported to be responsible for pesticidal activity in number of plants. And pesticides were ingested by the *Oryzaephilus surinamensis* and the compounds present in them such as oleic acid, elaidic acid isopropyl ester etc., was bound to the procathepsin L3 receptor present in the mid gut region of the pest that belongs to Silvanidae family. There was no mortality seen in control. The mortality count of the pest against all the three crude extracts is given below (Table 5.2). Percentage insect mortality was calculated using the Abbott's correction formula (Abbott, 1925).

$$\text{Abbott Value} = \frac{\text{Percentage of treated} - \text{Percentage of control}}{100 - \text{Percentage of control}} \times 100$$

Where Percentage of treated = Treated / total no of adult treated $\times 100$

Percentage of control = control / total no of adult control $\times 100$

Table 5.2 Pesticidal activity of *Manihot esculenta* against *Oryzaephilus surinamensis*

EXTRACT	CONCENTRATIONS (ppm)	ADULTICIDAL (%)	
		24h	48h
Chloroform	62.5	0.0±0.0	0.0±0.0
	125	0.0±0.0	0.0±0.0
	250	0.0±0.0	6±2.5
	500	12±2.0	32±2.0
	1000	28±2.0	60±3.2
Ethyl acetate	62.5	0.0±0.0	0.0±0.0
	125	0.0±0.0	0.0±0.0
	250	0.0±0.0	14±2.5
	500	8±2.0	34±2.5
	1000	44±2.5	62±2.0
Methanol	62.5	0.0±0.0	0.0±0.0
	125	0.0±0.0	14±2.5
	250	16±2.5	34±2.5
	500	32±.20	66±2.5
	1000	68±2.0	100±0.0

Mean of five replication ± SE



Fig: 5.8: Pesticidal bioassay

5.4. ANTI-OXIDANT ASSAY – DPPH free radical scavenging activity

DPPH Radical Scavenging. DPPH (1, 1-diphenyl-2-picrylhydrazil) analysis is one of the best-known, accurate and frequently employed methods for evaluating antioxidant activity. It is a stable free radical because of its spare electron delocalization over the whole molecule. The donation of H⁺ to the DPPH radicals made a corresponding change from violet color to pale yellow in the solution. The DPPH scavenging also made a proportionate decrease in its absorbance at 515nm (Bhuiyan et al, 2009). The absorbance value is shown below (Table 5.3). The IC₅₀ value is 117 µg/ml.

Table: 5.3: % of radical scavenging activity of methanolic extracts of *Manihot esculenta*

S NO	CONCENTRATION µg/ml	ANTIOXIDANT (%)
1	20	0.46
2	40	1.52
3	60	2.97
4	80	19.63
5	100	24.88
6	120	51.14

From the above absorbance value, % radical scavenging activity is calculated by the formulae:

$$\% \text{ inhibition of DPPH} = [\text{Control} - \text{Sample}/\text{Control}] \times 100$$

The values obtained were plotted in the graph (Fig 5.8) to calculate the IC₅₀ value (Table 5.3) of the methanolic extract of *Manihot esculenta*.

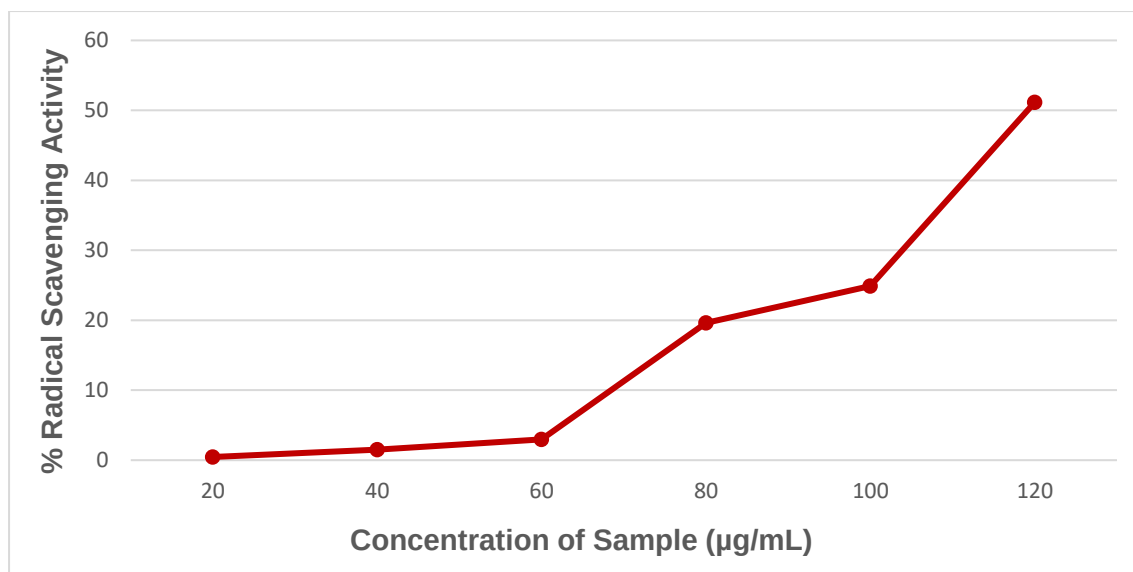


Fig: 5.9: graph of % radical scavenging activity

5.5. PURIFICATION OF CRUDE EXTRACTS

5.5.1. THIN LAYER CHROMATOGRAPHY

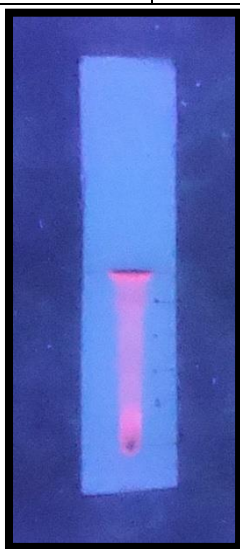
The methanolic extract of plant was run with hexane: ethyl acetate in the ratio of 1.5: 0.5, the ethyl acetate extract was run with hexane: ethyl acetate in the ratio of 1.5: 0.5 and chloroform extract was run with hexane: ethyl acetate in the ratio of 1.5: 0.5 these are kept undisturbed for 30 mins. The TLC plate was then visualized under UV-transilluminator and the bands were marked for calculation for R_f factor which are tabulated below (Table 5.4). More number of bands was for chloroform extract which showed the maximum number of compounds separated. This may be due to the presence of a greater number of less polar molecules present in the plant. Studies indicate that the compounds with larger R_f value indicate the presence of less polar compounds as they interact strongly with polar adsorbent (Gocan, 2002). Chloroform extract revealed larger R_f value having 0.97 respectively.

Table: 5.4: Rf values for TLC of plant solvent extracts

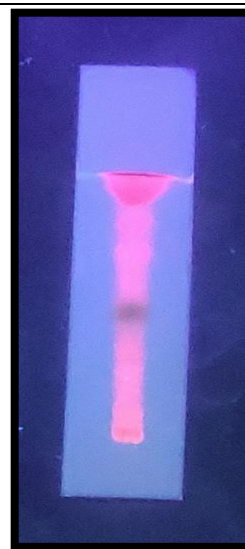
SOLVENTS	EXTRACTS
Methanol	B1 – 0.26
	B2 – 0.44
	B3 – 0.58
	B4 – 0.76
	B5 – 0.88
Ethyl acetate	B1 – 0.19
	B2 – 0.42
	B3 – 0.61
	B4 – 0.80
Chloroform	B1 – 0.13
	B2 – 0.23
	B3 – 0.36
	B4 – 0.56
	B5 – 0.71
	B6 – 0.81
	B7 – 0.97



1



2



3

Fig: 5.10: TLC Results (1. Methanolic extract, 2. Ethyl acetate extract, 3. Chloroform extract)

5.6 FTIR (Fourier Transform Infrared Spectroscopy)

FTIR analysis was done for methanolic extract, to identify the functional group present in the compound. The absorption band FTIR analysis at 3267 cm^{-1} indicates the stretching of O-H bonds. The absorption band of 2922 cm^{-1} , 2852 cm^{-1} , 2115 cm^{-1} indicates the C-H stretching of alkane group. The absorption band of 1599 cm^{-1} and 1514 cm^{-1} indicates the C=C stretching of alkene group. The absorption band of 1401 cm^{-1} corresponds to C-H bending of methylene group. The absorption band at 1165 cm^{-1} and 1046 cm^{-1} can be attributed to the stretching vibration of C-O of carboxyl group. Coates, 2006, have performed basic computer based spectral searching in which the absence of suitable reference database, it is still possible to effect a basic interpretation of the spectrum from the first principles, this first principles approach is used to find structural features of the molecules, like whether they are the backbone of the molecules or functional groups that is attached to the molecule, this produce characteristic and reproducible absorptions in spectrum. Through this information we can indicate whether there is backbone to structure, if so, whether the backbone consists of liner or branched chains. Finally the functional group present is identified. Therefore, the functional groups were identified by comparing the bands with the studies conducted by (Coates, 2006).

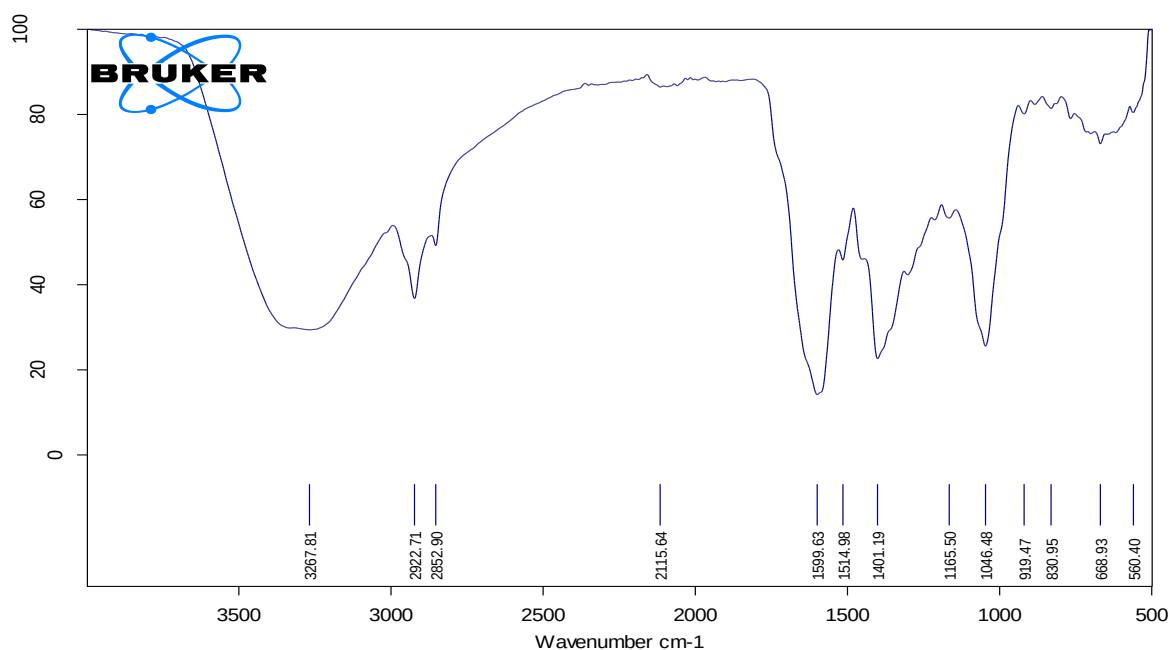


Fig: 5.11: FTIR spectrum of *Manihot esculenta* for methanolic extract

5.7. GAS CHROMATOGRAPHY- MASS SPECTROSCOPY ANALYSIS

The presence of different types of compounds was identified using GC-MS analysis. Literature study shows that Phytol Oleic acid and Elaidic acid, isopropyl ester, Flavone and 14-methyl-18-oxononadec-14-enoic acid methyl ester are responsible for pesticidal activity and other medicinal properties present in plant. The presence of the compounds found in the methanolic crude extract which indicates that this plant does have pesticidal property.

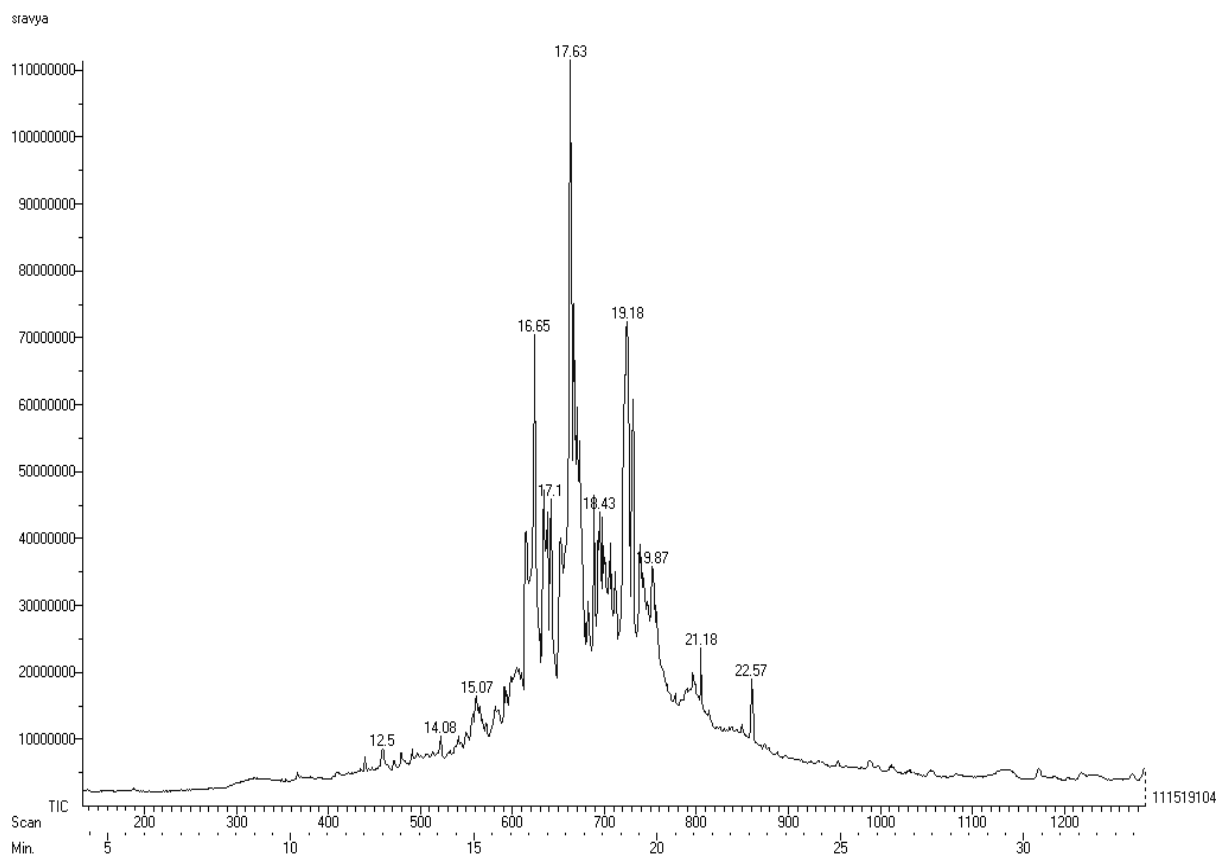
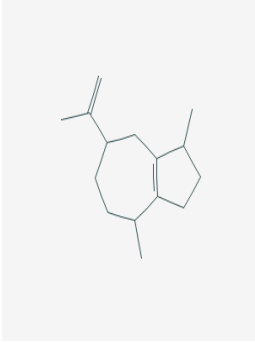
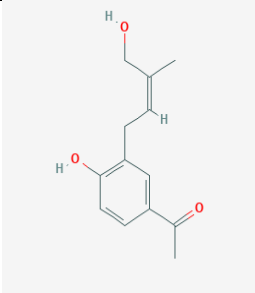
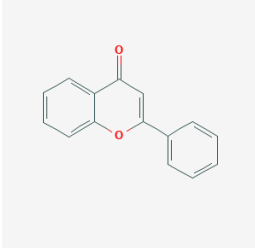
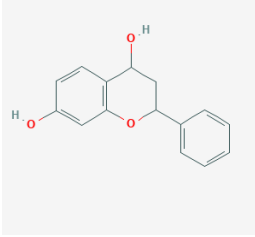
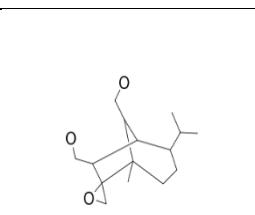
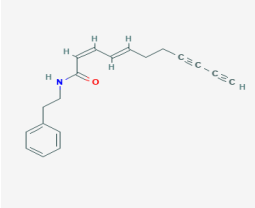
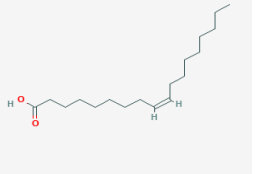
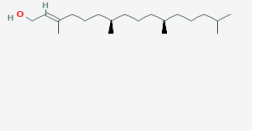
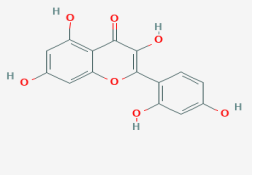
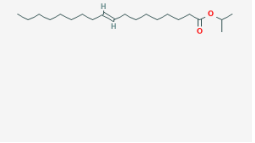
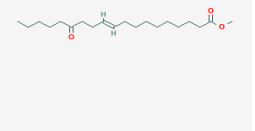


Fig: 5.12: Gas Chromatograph of methanolic extract of *Manihot esculenta*

Table: 5.5: GC-MS analysis of methanolic extract of *Manihot esculenta*

S NO	COMPOUND NAME	RT	COMPOUND STRUCTURE	MOLECULAR WEIGHT (g/mol)	MOLECULAR FORMULA
1	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7(1-methylethylidene)	12.5		204.35	C ₁₅ H ₂₄
2	3-[4-Hydroxyisopent-2(Z)-enyl]-4-hydroxyacetophenone	14.08		220.26	C ₁₃ H ₁₆ O ₃
3	Flavone	15.07		224.24	C ₁₅ H ₁₀ O ₂
4	2H-1-Benzopyran-4,7-diol, 3,4-dihydro-2-phenyl-	16.55		242.27	C ₁₅ H ₁₄ O ₃
5	Spiro-6-(bicyclo[3.2.1]octane)-2'-(oxirane), 7,8-di(hydroxymet	17.1		254.37	C ₁₅ H ₂₆ O ₃

	hyl)-5-methyl-2-isopropyl				
6	N-(2-phenylethyl)undeca-(2Z,4E)-diene-8,10-dynamide	17.63		277.4	C ₁₉ H ₁₉ NO
7	Oleic acid	18.43		282.5	C ₁₈ H ₃₄ O ₂
8	Phytol	19.18		296.5	C ₂₀ H ₄₀ O
9	Morin	19.87		302.23	C ₁₅ H ₁₀ O ₇
10	Elaidic acid, isopropyl ester	21.18		324.5	C ₂₁ H ₄₀ O ₂
11	14-Methyl-18-Oxononadec-14-enoic acid, methyl ester	22.57		324.5	C ₂₀ H ₃₆ O ₃

5.8. ANOVA ANALYSIS

Table: 5.6: ANOVA analysis

source of variation	SS	Df	MS	F	P-value	F critical
Rows	18488	14	1320.571	16.05604	0.000002	2.483725
Columns	1526.533	1	1526.533	18.56021	0.000722	4.6001
Error	1151.466	14	82.24761			
Total	21166	29				

P –value is less than 0.05 which is a predetermined value. Similarly, F-value (16.05/18.56) is greater than F critical value (2.48/4.60), indicating that the means are statistically significant. Hence, concentration of the biopesticides has a significant effect of the pests.

5.9 MOLECULAR DOCKING

Oleic acid Elaidic acid, isopropyl ester, Flavone and 14-methyl-18-oxononadec-14-enoic acid methyl ester are the compounds that is present in the methanolic extract crude, which is reported to exhibit the pesticidal activity according to the literature survey. Molecular docking was done between these compounds and procathepsin I3 RECEPTOR presents in the midgut region of the pest. The binding between the ligand and receptor was observed and a dock score was obtained for both Oleic acid and Elaidic acid, isopropyl ester, Flavone and 14-methyl-18-oxononadec-14-enoic acid methyl ester.

- Dock score for Oleic acid : -39.77.
- Dock score for Elaidic acid, isopropyl ester : -60.68
- Dock score for Flavone : +7.64
- Dock score for 14-methyl-18-oxononadec-14-enoic acid methyl ester : -17.25

The dock is used to quantify the predictive capability of a docking protocol. They are used to predict the binding affinity between two molecules after they have been docked. Studies indicate that the docking score of -10 or lower usually represent good binding and therefore, the above score which are less than -10 clearly reveal the higher binding affinity between the pesticide and the pest. The binding between the compounds present in the pesticide and the receptor present in the gut region of the pest is showed below. (Fig 5.12, Fig 5.13, Fig 5.14, Fig 5.15).

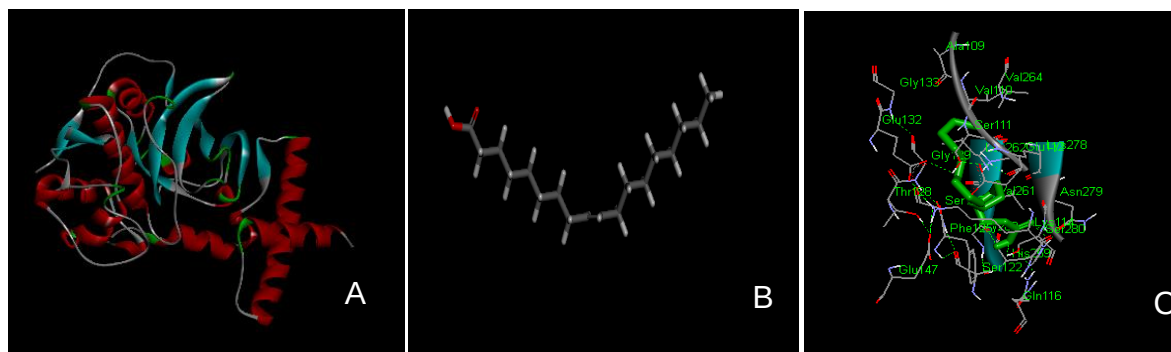


Fig 5.13 Molecular docking for Oleic acid. A- Procathepsin I3 RECEPTOR, B- Oleic acid (Ligand), C- Active binding site of ligand and protein.

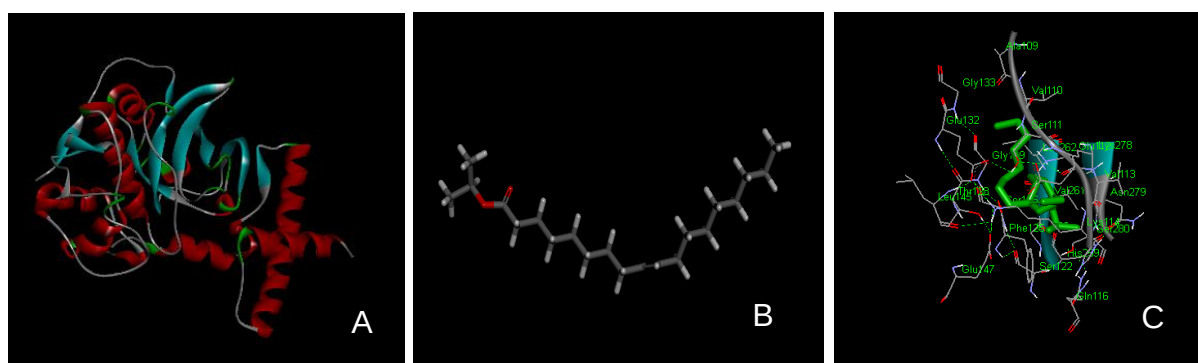


Fig 5.14 Molecular docking for Elaidic acid, isopropyl ester. A- Procathepsin L3 RECEPTOR, B- Elaidic acid, isopropyl ester (Ligand), C- Active binding site of ligand and protein.

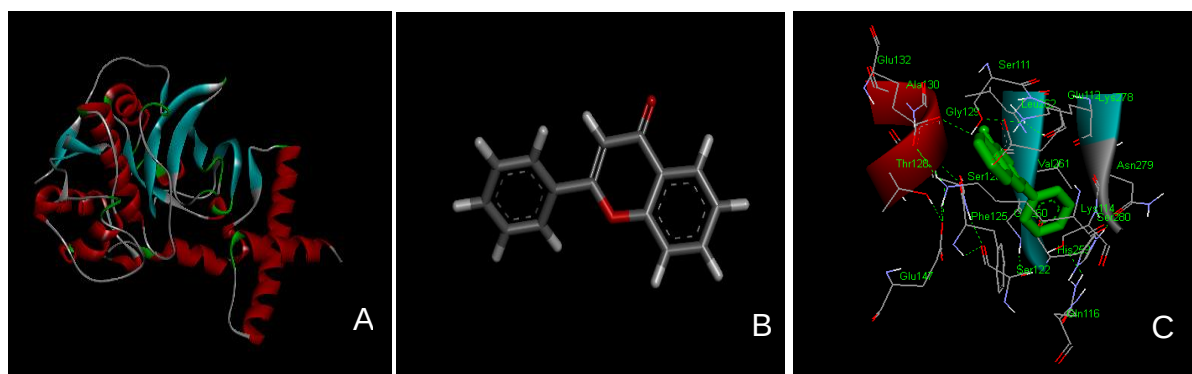


Fig 5.15 Molecular docking for Flavone. A- Procathepsin I3 RECEPTOR, B- FLAVONE (Ligand), C- Active binding site of ligand and protein.

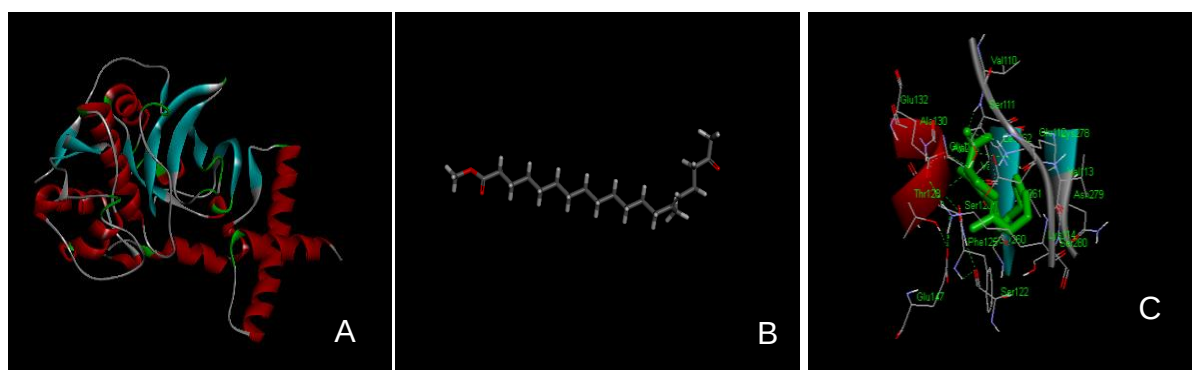


Fig: 5.16: Molecular docking for 14-methyl-18-oxononadec-14-enoic acid methyl ester. A- Procathepsin I3 RECEPTOR, B- 14-methyl-18-oxononadec-14-enoic acid methyl ester (Ligand), C- - Active binding site of ligand and protein.

CHAPTER – 6

SUMMARY AND CONCLUSION

6.1 SUMMARY

- The study was focused on efficient biopesticides from a plant and evaluating its activity against stored pest *Oryzaephilus surinamensis*.
- *Manihot esculenta* commonly called cassava were collected and analyzed for various tests and their pesticidal activity was evaluated against *Oryzaephilus surinamensis*.
- Fresh leaves of cassava were collected and shade dried for two weeks and subjected to maceration process.
- Three solvents were used for this maceration process methanol, ethyl acetate and chloroform, at the end of the process the resultant extract were left to dry in a plate.
- The extract in a plate was left to dry for two days to obtain the crude and were stored in a room temperature for further analysis.
- Preliminary screening of extracts was done through phytochemical analysis and anti-microbial tests.
- The pesticidal activity each solvent extract were assessed by exposing them into a glass vials coated with the solvent extract on the inner side of the vial.
- They were observed for 24 hr and 48 hr by observing the phenotypic changes and mortality count of *Oryzaephilus surinamensis*.
- Methanol extract was found to be effective against the pest (100% mortality rate).
- Antioxidant assay for methanol extract was performed and the IC 50 value is 117 µg/ml.
- TLC of three solvent extracts were done in order to find the appropriate solvent system for maximum purification of compounds.
- The methanol extract which was highly effective against were characterized using FTIR and GC-MS.
- ANOVA analysis was done and the concentration of the biopesticides has a significant effect of the pests.

- Molecular docking analysis was done for the compounds present in the methanol extract and two compounds four compounds present in the extract - Oleic acid, Elaidic acid, isopropyl ester, Flavone and 14-methyl-18-oxononadec-14-enoic acid methyl ester was found to bind with Procathepsin L3 Receptor present in the mid gut region of the pest with the dock score of -39.77, -60.68, +7.64 and -17.25 respectively. By comparing with the literature studies, it was found that the pesticides had good binding affinity with the pest.

6.2. CONCLUSION

The above data and study reveals that plant extracts of *Manihot esculenta* leaves in methanol solvent is highly toxic against adults *Oryzaephilus surinamensis*. The methanol extract of *Manihot esculenta* would be used as a potential grain protectant against *Oryzaephilus surinamensis*. it provides safer alternative than the chemical pesticides. Biopesticidal activity from *Manihot esculenta* using three solvents – methanol, ethyl acetate and chloroform was performed. Among the three solvents methanolic extract of *Manihot esculenta* showed the 100% mortality rate. By the means of GC-MS the compounds present in the methanolic crude extract was docked against the receptor present in the midgut region of the pest showed good binding affinity with the pest, therefore the plant *Manihot esculenta* has biopesticides properties. Unlike chemical pesticides, biopesticides are more targets specific and therefore, they intervene less with natural ecosystem. Therefore the methanolic crude extract of *Manihot esculenta* can be used as an alternative for chemical pesticides as it causes adverse effects on the environment.

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