Current Trends in Biotechnology and Pharmacy
Vol. 15 (5) 524-539, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2021.3s.50

Bioactive potential and pharmacological activity of *Psidium guajava*

Vijayanand S* and Kashmeera George
Department of Life Sciences
Kristu Jayanti College(Autonomous) Bengaluru
*Corresponding author: vijayanand78bio@rediffmail.com

Abstract

The present study, was aimed to extract the peroxidase enzyme from the leaves of Psidium guajava and optimized its activity at different temperatures, pH and substrate concentration. The activity of the Guava peroxidase was determined by enzyme assay and the specific activity of the enzyme was determined by protein estimation. The amount of the protein present in the Guava sample was found to be 7.0mg.The specific activity of the protein was 0.03u/ml/mg. Thermal inactivation was studied in 10mM Tris – Hcl buffer pH (8.0). The activity of Guava peroxidase was found stable in 3 different time intervals of 5, 10, and 15mins. Guava peroxidase protein profiling by SDS-PAGE revealed the molecular weight of leaf sample was 55KDa.The maximum activity of the Guava leaf peroxidase was on pH 5.0, temperature 70ºc. Further the study also demonstrates the antimicrobial potential of *Psidium guajava* leaves extract by using various solvents. The results indicate that ethanol is solvent of better choice than distilled water for the extraction. The observed inhibition of gram positive bacteria, *s.aureus* suggest that Guava possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using ethanol as the solvent. On the basis of the present finding*, P. guajava* leaves possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and/or diseases caused by *S. aureus*.

Keywords: Antimicrobial activity *Psidium Guajava, Staphylococcus aureus, Peroxidase*

Introduction

Nature has been a source of medicinal agents for thousands of years and as impressive number of modern drugs has been isolated from natural sources, many based on their use in traditional medicine. In India, the use of plants as medicine has been passed through generations, right from the Vedic period. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicine. Higher plants, as source of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient time. (Smitha Jain et al., 2010)

The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs. Plant sources play an important role in drug development programs in the pharmaceutical industry. Due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant Kingdome has forced our researchers to screen scientifically various traditional claims (Nair et al., 2004)

Plants have been used in treating human diseases for thousands of years. Some 60,000 years ago, it appears that Neanderthal man valued herbs as medicinal agents; this conclusion is based on a grave in Iran in which pollen grains of eight medicinal plants were found (Solecki and Shanidar ,1975)

The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines (Duke 1985). A 1997 survey showed that 23% of Canadians have used herbal medicines. In addition, as much as 25% of modern pharmaceutical drugs contain plant ingredients (Duke, 1993).There are a huge number of medicinal plants. It has been estimated that about 13,000 species of plants have been employed for at least a century as traditional medicines by various cultures around the world (Tyler, 1993)

The medicinal qualities of plants are of course due to chemicals. Plants synthesize many compounds called primary metabolites that are critical to their existence. These include proteins, fats, and carbohydrates that serve a variety of purposes indispensable for sustenance and reproduction, not only for the plants themselves, but also for animals that feed on them.

Plants also synthesize a dazzling array of additional components, called secondary metabolites, whose function has been debated. Many secondary metabolites are "antibiotic" in a broad sense, protecting the plants against fungi, bacteria, animals, and even other plants. Every plant species contains chemicals that can affect some animals or micro-organisms negatively, strongly supporting the interpretation that

secondary metabolites play a vital role in combating diseases and herbivores. “Plants have been a rich source of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defense against predation or infection” (Cox and Balick ,1994)

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies. Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others plants. Other plants their derivatives consider as important source for active ingredients which are used in aspirin and toothpaste. (Rasool Hassan, 2012)

Plants store food in various ways, either in roots, stems, leaves, fruits or seeds. The most important of these for humans are fruits and seeds. In this category are found the cereals and small grains, the legumes and the nuts. All contain large amounts of nutritive material and have proportionately low water content, which enhances their value for they can be stored and transported with ease. Roots, tubers, bulbs and other vegetables from the soil are next in importance as sources of our food and the lower animals as well. Their value is less because they contain much water. The leafy parts of plants, the greens, salad plants and other herbage vegetables contain comparatively little stored food. But they are necessary because of the vitamins and mineral salts they contain and the mechanical effect of their indigestible cellulose. This is true also of the fleshy fruits that may also contain various organic acids.

The use of medicinal plants as a source for relief from 130 drugs, all single chemical entities extracted from illness can be traced back over five millennia to written higher plants, or modified further synthetically. Plants are used medicinally in still widely used in ethno medicine around the world different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts the potential of higher plants as source for new is used for extract as raw drugs and they possess varied drugs is still largely unexplored. Among the estimated medicinal properties, The different parts used include 250,000-500,000 plant species, only a small percentage has root, stem, flower, fruit, twigs exudates and modified plant been investigated phytochemically and the fraction organs. While some of these raw drugs are collected in submitted to biological or pharmacological screening is smaller quantities by the local communities and folk even smaller. Thus, any phytochemical investigation of a healers for local used, many other raw drugs are collected given plant will reveal only a very narrow spectrum of its in larger quantities and traded in the market as the raw constituents. Historically pharmacological screening of material for many herbal industries, although compounds of natural or synthetic origin has been the hundreds of plant species have been tested for source of innumerable therapeutic agents. Random antimicrobial properties, the vast majority of have not screening as tool in discovering new biologically active been adequately evaluated.(Satish .S et al., 2008)

A peroxidase is one of a number of enzymes that act as catalysts to allow a variety of biological processes to take place. Specifically, they promote the oxidation of various compounds using naturally occurring peroxides, especially hydrogen peroxide (H_2O_2), which are reduced, forming water. Peroxides are created as byproducts of various biochemical reactions within organisms, but can cause damage as they are oxidizing agents. Peroxidase break these compounds down in to harmless substances by adding hydrogen, obtained from another molecule known as a donor molecule in a reduction-oxidation (redox) reaction in which the peroxide is reduced to form water, and the other molecule is oxidized. There are a large number of these enzymes, and they are found in plants and animals, including humans. (Kumar P et al., 2008)

Like all enzymes, peroxidases are very large, complex molecules with complicated shapes involving multiple folds. They come in a variety of types, some of which can use a wide variety of donor molecules and reduce a wide range of peroxides, and some of which are much more specific. Enzymes have an “active site,” which is the part of the molecule where the reaction takes place. This may be in an easily accessible part of the molecule, or it may be tucked away in a fold, where it can only be reached by a molecule of exactly the right shape. Horseradish peroxidase (HRP) is an example of an enzyme that can use a wide variety of donor molecules and peroxides. (Sing J et al., 2008)

Peroxidases and its effects on plants were analysed. Peroxidases are involved in many physiological processes in plants, involving responses to biotic and abiotic stresses and the biosynthesis of lignin. Lignin is a polymer responsible for rendering the plant stronger and more rigid and also making the cell walls hydrophobic. Peroxidase is involved in the polymerization of the precursors of lignin. They are also involved in the scavenging of Reactive Oxygen Species (ROS), which are partially reduced forms of atmospheric oxygen, highly reactive, and capable of causing oxidative damage to the cell. Peroxidases can be a source of hydrogen peroxide (H2O2) but also are capable of scavenging it. (Kinsley C, 2000).

Peroxidases have been used for various analytical applications in diagnostic kits, such as quantification of uric acid, glucose, cholesterol and lactose. Some novel applications of peroxidases suggested include treatment of waste water containing phenolic compounds (that are present in widely varying concentrations in the

wastewaters of oil refineries and numerous other industries, including the plastics, resins, textiles, iron, steel and forestry industries), synthesis of various aromatic chemicals and removal of peroxide from materials such as food stuffs and industrial waste. More recently some investigators have reported the decolorization and removal of textile dyes from polluted water and dyeing effluents by using soluble and immobilized peroxidases (Ghaemmaghami F, 2010).

Plant peroxidases are receiving increasing attention due to their extensive bio activation properties and potential applications in clinical, biochemical, biotechnological and related areas. Advances have recently been made in using them to synthesize, under mild and controlled conditions, chiral organic molecules, which are highly valuable compounds. They have also been successfully employed in the development of new bioanalytical tests, improved biosensors and in polymer synthesis (Alemzadeh I, 2010).

Multiple forms of peroxidase are widely distributed in plants, microbes and animal tissue representing a huge family of heme containing enzyme and have been used in great number of analytical application such as clinical diagnosis (blood sugar and cholesterol), immunoassays (ELISA kit), biosensor construction , food processing and food storage, treatment of waste water containing phenols and aromatic amines, bioleaching process, lignin degradation in fuel, production of dimeric alkaloids oxidation, biotransformation of organic compounds. Plant peroxidase is found in tonoplast and plasmalemma, inside and outside the cell wall and it occur in the soluble, as well as, ionic ally bound forms on the cellular walls. Peroxidase has been implicated in metabolic process such as ethylene biogenesis, cell development and membrane integrity, deafens mechanism towards pathogen and various a biotic stresses, including metal ions , salt, UV stress , air pollution damage , cold tolerance , control of cell elongation , generation of reactive oxygen species, hydrogen peroxide scavenging . This enzyme can participate in a number of oxidation and bio degradation reactions associated with changes of flavor, color, texture and the nutritional quality. (Rao P et al., 2007)

1.1: PSIDIUM GUAJAVA (GUAVA PLANT)

Figure 1: Psidium guajava

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Order	:	Myrtales
Family	:	Myrtaceae
Subfamily	:	Myrtoideae
Genus	:	Psidium
Species	:	P.guajava
Botanical name	:	Psidium guajava

Guavas are cultivated throughout the tropical and subtropical areas of Africa, South Asia, South East Asia, The Caribbean, and North America. Guava can grow in both humid and dry tropical or subtropical climates. Guava is cultivated on varied types of soils- heavy clay to very light sandy soils. Good quality guavas are produced in river-basins. It tolerates a soil pH of 4.5- 8.2. Maximum concentration of its feeding roots is available up to 25 cm soil depth. Guavas cannot tolerate frost. Guavas are partly deciduous, shallow-rooted shrubs or small tree that, in the Darwin area, grows up to 5 meters with spreading branches. It has white flowers and edible round or pear-shaped sweet fruits usually 5-10 cm in diameter. .The fruit has a thin peel, usually pale green or yellow when mature. The flesh of some varieties is hard (crunchy like an apple) other cultivars are white or pinkish in flesh color and soft when ripe with a strong, very characteristic fragrant scent. The fruit centre core contains many small hard seeds. For the Darwin region, the variety of 'Northern Gold' as planted in the Community Orchard can be recommended. Some others are Thai White (Glom Sali), Common and Cherry. There are seedless varieties known but they generally lack flavor. (Robert E, 1992)

Guavas are low in calories and fats but contain several vital vitamins, minerals, and antioxidant poly-phenolic and flavonoid compounds that play a pivotal role in prevention of cancers, anti-aging, immune-booster, etc.

The fruit is very rich source of soluble dietary fiber**,** which makes it a good bulk laxative. The fiber content helps protect the colon mucous membrane by decreasing exposure time to toxins as well as binding to cancer-causing chemicals in the colon. (Curtis.J, 1997).Guava-fruit is an excellent source of antioxidant vitamin-C. 100 g fresh fruit provides 228 mg of this vitamin, more than three times the DRI (daily-recommended intake). Outer thick rind contains exceptionally higher levels of vitamin C than central pulp.

Scientific studies shown that regular consumption of fruits rich in vitamin C helps the body develop resistance against infectious agents and scavenge cancer causing harmful free radicals from the body. Further, the vitamin is required for collagen synthesis within the body. Collagen is the main structural protein in the human body

required for maintaining the integrity of blood vessels, skin, organs, and bones.

The fruit is a very good source of Vitamin-A, and flavonoids like beta-carotene, lycopene, lutein and cryptoxanthin. The compounds are known to have antioxidant properties and are essential for optimum health. Further, vitamin-A is also required for maintaining healthy mucus membranes and skin. Consumption of natural fruits rich in carotene is known to protect from lung and oral cavity cancers.Fresh fruit is a very rich source of potassium. It contains more potassium than other fruits like banana weight per weight. Potassium is an important component of cell and body fluids that helps controlling heart rate and blood pressure.

Further, the fruit is also a moderate source of B-complex vitamins such as pantothenic acid, niacin, vitamin-B6 (pyridoxine), vitamin E and K, as well as minerals like magnesium, copper, and manganese. Manganese is used by the body as a co-factor for the antioxidant enzyme, superoxide dismutase. Copper is required for the production of red blood cells. The Guava fresh leaf extract (decoction) is used to treat digestive disorders like diarrhea and vomiting whereas application of powdered leaves is believed to have soothing effect in rheumatic pains. (MacAdam, 2000)(Table 1)

The castor oil plant is a species of flowering plant in the spurge family. The evolution of castor and its relation to other species are currently being studied using modern genetic tool. An alcoholic extract of the leaf was shown, in lab rats, to protect the liver from damage from certain poisons. Metabolic extracts of the leaves of Ricinus communis were used in antimicrobial testing against eight pathogenic bacteria in rats and showed antimicrobial properties. The extract was not toxic. At high doses mice quickly died. Antihistamine and anti-inflammatory properties were found in ethanolic extract of Ricinus communis root bark. (Jennifer, 1994)

Castor seed is the source of castor oil, which has a number of uses. Castor oil is a viscous, pale yellow, nonvolatile and nondrying oil with a bland taste. It has good shelf life as compared to other vegetable oils. The seeds contain 40 to 60% oil that is rich in triglycerides mainly ricinolein a toxic alkaloid ricinine and very toxic albumen called ricin. The seed coat contains ricin, a poison which is present in lower concentrations throughout the plant. Riccinus communis has not only medicinal value but it also has great promises in the field of biodiesel production. It is inexpensive and environment friendly. There are different varieties of castor oil bean and on the average they contain 46-55% oil by weight. (Nelson et al., 1998)

Cabbage peroxidase is a relatively poorly studied enzyme. It is known that this heterogeneous heme protein presents quite significant heat stability; optimum activity at neutral (6–8) pH values and is suitable for effective immobilization. These properties make cabbage peroxidases highly promising for biotechnological applications, especially for constructions of stable and highly effective bioelectrocatalytic systems for oxygen reduction, with respect to e.g. potential applications in biofuel cells, as shown for other peroxidases. Moreover, cabbage heads are abundant and very cheap enzymatic source. However, it still lacks more detailed study, especially concerning storage stability or optical and electrochemical properties of these oxidize. (Bullen et al., 2006; Kulesza et al., 2007)

Among the other plant PODs; soybean peroxidase (SBP) is an inexpensive by- product of soybean seed hulls. It can be detected in the root, leaf and seed hulls of the soybean. The POD obtained from seed hulls contains the highest activity compared to the POD obtained from other parts of the soybean plant. SBP's marketed usages range from medical diagnostic tests to removal of chlorine-containing pollutants from industrial wastewater. An enzyme from soybean hulls is applied as a replacement for formaldehyde in adhesives, abrasives, protective coatings, and other products. Applying organic solvents for enzymatic reactions has several advantages including increased solubility of organic substrates, and shifting the hydrodynamic equilibria to favor synthesis over hydrolysis. However, quite a few reports are available on the biocatalytic characterization of SBP. Regarding its low price and potential applications, much more attention should be paid to SBP's biocatalytic efficacy in aqueous as well as in organic solvents, which is the aim of this investigation. (Motamed S, 2010)

Horseradish is a perennial root crop of the Crucifer family. It is one of the oldest known condiments, valued for its extremely pungent, fleshy roots (Rhodes et al., 1965)

Horseradish peroxidase (HRP) is isolated from horseradish roots (Amoracia rusticana) and belongs to the ferroprotoporphyrin group of peroxidases. It is used in biochemistry applications such as western blots, ELISA and Immuno histochemistry. Horseradish peroxidase is used to amplify a weak signal and increase detects ability of a target molecule, such as a protein. Product P8375, type VI, is an essentially salt free lyophilized powder. It is commonly used to determine amounts of glucose and peroxides in solution. It has been used to study sensory input of cervical spinal cord neurons. The enzyme has been used to develop a thermo stable soybean peroxidase-based biosensor by cross-linking and electrically 'wiring' the enzyme through a red ox-conducting hydro gel to a glassy carbon electrode. It has also been used to assay the oxidation of low density lipoprotein. (Kumar J J, 2000)

HRP readily combines with hydrogen peroxide (H_2O_2) and the resultant [HRP-H_2O_2] complex can oxidize a wide variety of hydrogen donors. The optimal pH is 6.0-6.5 and the enzyme is most stable in the pH

range of 5.0-9.0. HRP can be conjugated to antibodies by several different methods including glutaraldehyde, periodate oxidation, through disulfide bonds, and also via amino and thiol directed cross-linkers. It is smaller and more stable than the enzyme labels β-galactosidase and alkaline phosphatase and hence, it is the most desired label. Also, its glycosylation leads to lower non-specific binding. When incubated with a substrate, horseradish peroxidase produces a coloured, fluorimetric, or luminescent derivative of the labeled molecule, allowing quantification (Nair S, 2010)

Sweet pepper is considered to be a sensitive crop to water stress, as reductions in soil water availability or soil water excess jeopardize its normal development. According to (Carvalho et al. 2001) the amount of water made available to sweet pepper is a limiting factor for the growth and development of plants, causing stress conditions that limit their growth. Peroxidases are enzymes naturally found in plants. They are composed of 25% carbohydrates that protect them from proteolytic degradation and stabilize their protein structure (Siegel, 1995).(Gaspar et al. 1985) described peroxidases as isoenzymes associated to changes in physiological processes of plants under stress(Chang and Kao 1998) considered that the high enzyme activity in stressed plants may indicate the ability of certain genotypes to degrade toxic substances, such as free radicals released under such conditions.(Jadoski S O et al.,2001)

The guava (Psidium guajava) is a phytotherapic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases. The many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions. This plant has also been used for the controlling of life-changing conditions such as diabetes, hypertension, and obesity. (Bipul Biswas, 2013)

Bipul.Biswas et al carried out a study on antimicrobial activity of leaf extract of guava.they carried out their work with 2 grm –positive and 2 gram –negative bacteria which are some of foodborne and spoilage bacteria. The guava leaves were extracted in four different solvents of increasing polarities (hexane, methanol, ethanol, and water). The efficacy of these extracts was tested against those bacteria through a well-diffusion method employing 50 µL leaf-extract solution per well. According to the findings of the antibacterial assay, the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts. The methanol extract had an antibacterial activity with mean zones of inhibition of 8.27 and 12.3 mm, and the ethanol extract had a mean zone of inhibition of 6.11 and 11.0 mm against B. cereus and S. aureus, respectively. On the basis of the present finding, guava leaf-extract might be a good candidate in the search for a natural antimicrobial agent. (Bipul Biswas et al., 2013)

Samuel Mathew et al (Samuel mathew, 1998) have done a study on medicinal plants.the classification. Cultivation, utilization quality evaluation is done by J. Thomas et al (J.Thomas et al, 1998). The medicinal uses of local flora were carried out by Gerald B. Hammond et al. (Gerald B. Hammond et al., 1998).

According to Sakhrov et al, the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts. The methanol extract had an antibacterial activity with mean zones of inhibition of 8.27 and 12.3 mm, and the ethanol extract had a mean zone of inhibition of 6.11 and 11.0 mm against B. cereus and S. aureus, respectively. On the basis of the present finding, guava leaf-extract might be a good candidate in the search for a natural antimicrobial agent. (Sakharov et al., 2000)

Test of antimicrobial activities using diffusion agar method. The results showed levels of tannins in leaves of guava with ethanol 30 %, which is 2.351 mg /g, ethanol 50 % is 1.728 mg g, ethanol 70 % is 1.835 mg / g. The best solvent to extract the highest levels of tannins with ethanol 30 % by value of tannin levels 2.351mg /g. (Meigy Nelce Mailoa et al., 2014)

Sakhrov et al,2000 carried out a study on African oil palm .they found that, Palm peroxidase possesses uniquely high thermostability and is more stable in organic solvents than horseradish peroxidase .(Sakhrov et al.,2000)

Peroxidase has been assayed by a chronometric method involving the coupled reaction of ascorbic acid with the product of the enzymatic action on benzidine. Measurements of the activities of horseradish and tea peroxidase by this and two other methods, involving respectively pyrogallol and o-dianisidine, are compared. It is claimed that the chronometric method is relatively simple, rapid andaccurate. The method can be used in the presence of poly phenol oxidases. (Gregory, 1996)

The stability of crude peroxidase incubated for different period of time and at different pH and temperatures of four vegetables (potato, carrot, eggplant and tomato) was investigated by Suha et al. (Suha et al., 2013)

Peroxidases of high activity were extracted at pH 5.0 from potato and tomato while those of carrot and eggplant were extracted at pH 6.0. Potato tuber contained higher level of peroxidase whereas carrot had lower levels at all pH values. The results showed that the rate of loss of peroxidase activity from the vegetables increased with both increase in temperature and heating

time. Biphasic inactivation curves were observed for the enzymes extracted from all samples, where the initial heat inactivation is rapid followed by much slower inactivation periods. (Babiker, 2013)

Potato peroxidase was observed to be more stable to heat. A less severe heat treatment is required to inactivate carrot, egg plant and tomato peroxidases. (Babiker, 2013)

Heat inactivation characteristics differed for acidic (A), neutral (N), and basic (B) broccoli peroxidase.At 65 °C, A was the most heat stable followed by N and B (Tipawan Thongsook B et al., 2005)

The denaturation temperature allowing the maximum reactivation was 90 °C for A and horseradish peroxidase (HRP) and 70 and 80 °C for B and N, respectively. In all cases, heat treatment at low temperatures for long times prevented reactivation of the heated enzymes. (Dianel M. Barrett, 2005)

The thermal stability of different POD forms was tested at different temperature by Lucrezia Sergio et al. The leaf POD forms showed greater heat stability compared to the head ones. . The heat sensitivity of three POD forms was different: the bound forms were characterized by greater heat stability than the soluble form. (Lucrezia Sergio et al., 2006)

The use of medicinal plants was done by Satish et al. Plants are used medicinally in still widely used in ethno medicine around the world different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts the potential of higher plants as source for new is used for extract as raw drugs and they possess varied drugs is still largely unexplored. Among the estimated medicinal properties, The different parts used include 250,000-500,000 plant species, only a small percentage has root, stem, flower, fruit, twigs exudates and modified plant been investigated phytochemically and the fraction organs.(Satish et al.,2008)

Historically pharmacological screening of material for many herbal industries, although compounds of natural or synthetic origin has been the hundreds of plant species have been tested for source of innumerable therapeutic agents. Random antimicrobial properties, the vast majority of have not screening as tool in discovering new biologically active been adequately evaluated. (Satish et al., 2008)

Peroxidase has been implicated in metabolic process such as ethylene biogenesis, cell development and membrane integrity, deafens mechanism towards pathogen and various a biotic stresses, including metal ions , salt, UV stress , air pollution damage , cold tolerance , control of cell elongation , generation of reactive oxygen species, hydrogen peroxide scavenging.(Rao D P et al.,2007)

María D. Groppa et al have done a study on activity and expression of peroxidases from sunflower. The isozyme patterns were tissue-specific. Little changes were found in isozyme patterns as a function of time, except for cotyledons which were the only organs to become senescent throughout our study. Only cationic isozymes were found in seeds and seedlings up to the fourth day after planting. Anionic isozymes began to appear from this day on and cationic isozymes began to disappear. This “switch” in isozyme expression was coincident with the appearance of the root and with a change in substrate specificity. (María D. Groppa et al., 1999)

Bania and Rita Mahanta have done a study on evaluation of peroxidases from various plant sources. Optimum pH and temperature for all the three plant peroxidases were also determined. B. oleraceae capitata var. Alba L had shown high thermal stability. These peroxidases may proof as a highly promising enzyme for practical application in biotechnological field. (Rita Mahanta, 2012)

Anna Belcarz et al did work on spring cabbage peroxidases – Potential tool in biocatalysisand bioelectrocatalysis. The best substrate for Px-cat

$$\text{Volume of Guava peroxidase activity (U / ml)} = \frac{\text{OD at 450 / min} \times 4 \times Vt \times \text{dilution factor}}{e \times Vs}$$

was pyrogallol and for Px-ani–o-dianisidine .Px-cat immobilized on polyanionic PyBA modified carbon electrode was found to produce linear repetitive signals upon consecutive additions of hydrogen peroxide during at least 1-week period and to work effectively under buffered and non-buffered conditions. These properties were comparable with those of commercially available horseradish peroxidase. Stability of the hybrid bioelectrocatalytic film and low costs of extraction and partial purification of Px-cat make it a highly promising enzyme for practical applications, including construction of bioelectrodes. (Anna Belcarz, 2007)

Palvannan T et al have done a study on effect of pH on peroxidase enzyme, effect of temperature on peroxidase enzyme and effect of substrate concentration on peroxidase enzyme.they find out the maximum activity of the perixidase enzyme (Palvannan T et al., 2005)

Mohammed A. Al-Fredan peroxidase activity in male and female plants of date palm (phoenix dactylifera l.) growing in a studied about the l-hassa, saudi arabia in vitro. A male palm and two female date palms (Gur-yellow fruited cultivar and Khunaizi- red fruited cultivar) were used in the study. Results revealed different leaf

isozyme patterns for the studied genotypes of date palm. The genotypes showed specific reliable mobility values for each isozyme band. Presence or absence of PRO 1 was identified as a reliable marker which distinguished male palms. However, further studies may be recommended to justify this conclusion. Diversity index and genetic distances were calculated as a measure of comparative relatedness among various genotypes. The values obtained for similarity coefficient showed wide diversity between male and female palms. The effect of some kinetic parameters such as buffer concentration, pH, and temperature and substrate specificity on peroxidase activity was also investigated. (Mohammed A. Al-Fredan, 2013)

$$\text{Volume of Guava peroxidase activity (U / ml)} = \frac{\text{OD at 450 / min} \times 4 \times \text{Vt} \times \text{dilution factor}}{\text{e} \times \text{Vs}}$$

Galina G. Zhadan et al have done a study on isolation and classification of peroxidases and thermostability of oil palm peroxidases.the data obtained in this investigation show that thermal denaturation of palm peroxidase is an irreversible process, under kinetic control.(Galina G. Zhadan et al.,2002)

Seed Coat Soybean Peroxidase: Extraction and Biocatalytic Properties Determination was done by F. Ghaemmagham et al. Plants or plant food wastes have been given much less attention or even disregarded. In some instances, however, oxidative enzymes from residual plant tissues have been shown to effectively degrade recalcitrant pollutants. Soybean seed coat peroxidase (SBP) is an inexpensive oxidoreductive enzyme and could be potentially used to oxidize/ polymerize various organic pollutants of the industrial and petrochemical wastes. The catalytic properties of SBP are retained under a wide range of pH and at elevated temperatures. (Ghaemmaghami F et al., 2000)

$$\text{Volume of Guava peroxidase activity (U / ml)} = \frac{\text{OD at 450 / min} \times 4 \times \text{Vt} \times \text{dilution factor}}{\text{e} \times \text{Vs}}$$

Materials and Methods

SAMPLE COLLECTION

Leaves of guava were collected from the village area of narayanpura, kaenatka, India, and it is authenticated by botanist, life science department, kristu Jayanti College, Bangalore.

EXTRACTION OF THE SAMPLE

The Guava leaves were collected and purity was maintained. 25gms of fresh Goa leaves were washed with distilled water and homogenized in mortar with 125ml of 0.1M phosphate buffer of [pH 6] ,homogenate was rapidly filtered through six layers of cheese cloth .The filtrate was centrifuged at 12000rpm for 30 min (simple extraction method). The supernatant was taken and labeled as crude extract .The resultant extract which contain the enzyme was used for further studies. (Kamale M et al., 2008)

ENZYME ACTIVITY

DETERMINATION OF GUAVA PEROXIDASE ACTIVITY

Peroxidase activity was determined calorimetrically**:** 10µl of enzyme solution was added to 2 ml of 100mM citrate phosphate buffer [pH 5.5], containing 18.2mM guaiacol and 4.4mM H_2O_2 as substrate. The mixture was incubated for 5min at room temperature to determine activity and specific activity of peroxidase enzyme .The absorbance change at 470nm was monitored at 25^0c. The specific activity was expressed as units of activity per mg of protein .(Galaev I YU et al., 2000)

DETERMINATION OF SPECIFIC ACTIVITY OF GUAVA PEROXIDASE

ESTIMATION OF PROTEIN BY BRADFORD METHOD

Pipette out 0.1ml to 0.5ml of working standard solution in to a series of test tubes marked as S_1-S_5 respectively. Make up the given unknown sample to 100ml with chilled water.1ml of this unknown solution is added to the test marked as T_1 and T_2.To all the tubes add distilled water to make up the volume to 1ml.Take 1ml of distilled water in another tube to serve as blank. Pipette out 5ml of diluted dye reagent in to all the tubes and keep at room temperature at least 5 min but no longer than 30 min. Measure the blue color developed calorimetrically at 590nm. Intensity of the color formed is directly proportional to the amount of protein present in it. Draw standard graph by plotting the concentration of protein on X-axis and optical density reading on Y-axis. Find out the concentration of the unknown from the graph and calculate protein content in given sample. (Sadasivam S and Manickam A, 1996)

THERMAL INACTIVATION OF GUAVA PEROXIDASE ENZYME

Thermal inactivation of Guava peroxidase was

Figure 1: Psidium guajava

Figure 2: Crude extract from the Guava leaf

Table 1: List of plants possess peroxidase activity

Serial number	Plant possess peroxidase activity	Reference
1	Castor	P. Kumar(2008)
2	Cabbage	Chmielnicka. J(1966)
3	Soybean	Kamal AJK et al(2000)
4	Horse radish	Davis DM et al(1976)
5	Oil palm	J.C. Areza et al(2000)
6	Pepper plant	Arnnok.P et al(2010)

2.1: YIELD PERCENTAGE OF GUAVA LEAF EXTRACT

Table 2.1: Extraction of the Guava leaf sample

Serial number	Name of the plant	Weight of the sample	Solvents	% of yield
1	Psidium gujava	25g	Phosphate buffer (125ml)	22.4%

DETERMINATION OF GUAVA PEROXIDASE ENZYME

Table 3: Determination of standard activity of hydrogen peroxide

Serial number	Reagents required	Test tubes				
		S_1	S_2	S_3	S_4	S_5
1	Phospate buffer (ml)	2ml	2ml	2ml	2ml	2ml
2	Guaicol (g)	0.225g	0.225g	0,225g	0.225g	0.225g
3	Hydrogen peroxide(µl)	10µl	10µl	10µl	10µl	10µl
4	Concentration	10	20	30	40	50
5	ODat470 nm	0.5	0.10	0.16	0.20	0.20

Table 3.1: Determination of activity of Guava peroxidase enzyme

Serial number	Contents	Test		OD at 470nm
1	Phosphate buffer (ml)	2ml	Incubation for 5 min at room temperature	0.23
2	Guaiacol (g)	0.225g		
3	Hydrogen peroxide (g)	0.014g		
4	Enzyme solution (µl)	10µl		

DETRMINATION OF SPECIFIC ACTIVITY OF GUAVA PEROXIDASE BY BRADFORD METHOD

Table 3.1.1: determination of specific activity of Guava peroxidase

Serial number	**Reagents required (ml)**	**B**	**S_1**	**S_2**	**S_3**	**S_4**	**S_5**	**T**
1	**Bovine serum albumin(ml)**	**-**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**-**
2	**Unknown solution(ml)**	**-**	**-**	**-**	**-**	**-**	**-**	**1ml**
3	**Distilled water(ml)**	**1**	**0.9**	**0.8**	**0.7**	**0.6**	**0.5**	**-**
4	**Concentration in µg**		**20**	**40**	**60**	**80**	**100**	
5	**Dye (ml)**	**<**	**5ml**		**>**			
OD at 595nm		**0.00**	**0.25**	**0.29**	**0.43**	**0.50**	**0.53**	**0.50**

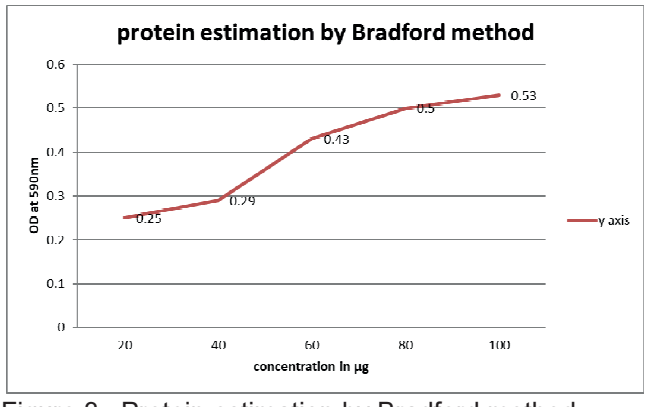


Figure 3: Protein estimation by Bradford method.

THERMAL INACTIVATON OF GUAVA PEROXIDASE ENZYME

Table 4.1: Determination of thermo stability of the Guava peroxidase enzyme

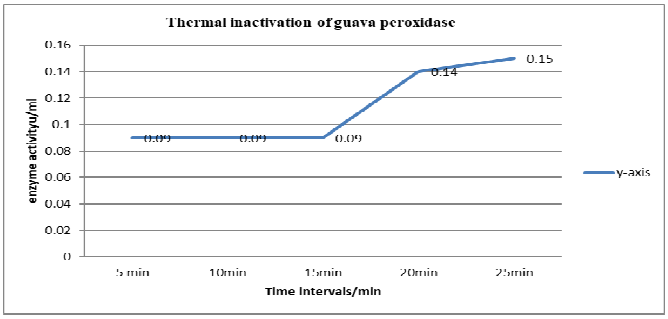
Serial number	Reagents	Test	Heat up to 80^0c
1	Tris – Hcl	3ml	
2	Enzyme source	10µl	

Serial number	Test	Volume of heated sample		Time interval	OD at 470nm
1	T_1	1ml	Incubation at 25^0c for 60min	5min	0.01
2	T_2	1ml		10min	0.02
3	T_3	1ml		15min	0.03
4	T_4	1ml		20min	0.06
5	T_5	1ml		25min	0.08

Table 4.2: determination of activity of Guava peroxidase on diffeternt time intervals

Time interval (min)	Activity of peroxidase enzyme(U/ml)
5min	0.09
10min	0.09
15min	0.09
20min	0.14
25min	0.15

Figure 4: Thermal stability of Guava peroxidase enzyme



OPTIMIZATIZATION

EFFECT OF PH ON GUAVA PEROXIDASE ENZYME

Table 5.1: Effect of pH on guava peroxidase enzyme

Respective buffer Acetate buffer	Volume of buffer (ml)	Volume of enzyme (ml)	Volume of guaiacol (ml)	Volume of hydrogen peroxide (ml)	OD at 450 for every 15sec up to 3 min						
					15 (s)	30 (s)	45 (s)	60 (s)	1 m	2m	3m
3.6	1ml	1ml	1ml	1ml	.04	.03	.04	.4	.08	.08	.08
4.2	1ml	1ml	1ml	1ml	.01	.06	.07	.10	.10	.10	.11
5.0	1ml	1ml	1ml	1ml	.26	.27	.28	.30	.31	.31	.33
5.6 Phosphate buffer	1ml	1ml	1ml	1ml	.18	.19	.19	.20	.21	.21	.22
6.0	1ml	1ml	1ml	1ml	.10	.15	.20	.25	.30	.30	.31
6.5	1ml	1ml	1ml	1ml	.15	.16	.20	.25	.30	.37	.40
7.5	1ml	1ml	1ml	1ml	.10	.12	.16	.20	.20	.25	.25
8.0	1ml	1ml	1ml	1ml	.20	.27	.30	.32	.33	.35	.36

studied in 10mM tris - Hcl, PH 8.0. After heating of the buffer [3ml] up to 80^0c, 10µl of peroxidase sample was added and the solution was incubated at the same temperature. Aliquots of the enzyme solution were removed at different times and incubated for 60min at 25^0c to restore the activity lost due to the reversible inactivation. Finally the activity was determined in each sample.the stability of the enzyme was expressed as percentage residual enzyme activity.the residual enzyme activity was measured as described in the peroxidase assay section. (Castillo.L et al., 2000)

Where,

Vt = final volime of reaction mixture (ml)

Vs = sample volume

e = micro molar extinction co- efficient of tetra guaiacol = 25.5

4 =derived from unit definition and principle

OPTIMIZATION OF GUAVA PEROXIDASE ENZYME

3.2.5.1: ENZYME SOURCE

Weigh 5g of leaf extract and added to the ice cold 0.1M sodium acetate buffer pH [4.2]. Strain the contents through muslin cloth and centrifuge at 15000rpm for 10 min to remove the debris. The supernatant is used as the source of enzyme.(Palvannan T,2005)

EFFECT OF PH ON GUAVA PEROXIDASE ACTIVITY

Set up a series of test tubes, each containing 0.1M buffer of the following pH values: 3.6, 4.2, 5.0, 5.6, 6.0, 6.5, 7.5, and 8.0. Start the experiment by adding 1ml of enzyme extract to the tube containing 0.1M acetate buffer pH [3.6]. Initiate the reaction by the addition of 1ml of guaiacol and 1ml of substrate hydrogen peroxide. Mix the contents by inversion and inset the cuvette in to the calorimeter. Take the reading for every 15sec up to 3 min at 450nm. Repeat the step 2-5 for each of indicated pH value. Plot the data in graph. (Palvannan T, 2005)

Where,

Vt = final volime of reaction mixture (ml)

Vs = sample volume

e = micro molar extinction co- efficient of tetra guaiacol = 25.5

4=derived from unit definition and principle

EFFECT OF TEMPERATURE ON GUAVA PEROXIDASE ACTIVITY

Set up a series of test tubes each containing 0.1M sodium acetate buffer pH 5.0 Place the tubes in an ice bath or incubator adjusted to the following temperatures 4, 15, 37, 50, and 70^0c. Add 1ml of enzyme extract to each of the tubes. Equilibrate the tubes at the respective temperatures for 2 min. Start the experiment with the tube kept at 4^0c. Adjust the calorimeter to 450 nm and blank properly. Add the enzyme 1ml in to the cuvette and begin the reaction by the addition of 1ml of guaiacol and 1ml of hydrogen peroxide. Mix the contents thoroughly. Repeat the steps 3-5 for each of indicated temperatures, and plot the data in the graph. (Shanmugam S, 2005)

Where,

Vt = final volime of reaction mixture (ml)

Vs = sample volume

e = micro molar extinction co- efficient of tetra

guaiacol = 25.5

4=derived from unit definition and principle

EFFECET OF SUBSTRATE CONCENTRATION ON GUAVA PEROXIDASE

Set up a series of test tubes, each containing 1ml of buffer, 1ml of enzyme extract, 1ml of guaiacol. Add 1ml of hydrogen peroxide to the 1st tube. Respective buffer pH [5.0] has taken as blank. The OD was taken at 450nm for every 15sec up to 3min. Repeat the experiments for each of tubes at a different substrate concentration [2ml- 5ml] with data collected determine the relationship between the velocity of the reaction (v) and the substrate concentration (s) at the different substrate concentration. Draw both Michaelis-Menton and Lineveaver-Burke graphs for data collected. (T.Satish kumar, 2005)

Where,

Vt = final volime of reaction mixture (ml)

Vs = sample volume

e = micro molar extinction co- efficient of tetra guaiacol = 25.5

4 =derived from unit definition and principle

PROTEIN PROFILE FOR GUAVA PEROXIDASE ENZYME

PROTEIN ISOLATION FOR SDS – PAGE ANALYSIS

Place 5g of collected leaf tissue in a mortar and pestle. Add 10ml of QB solution in to it. Grind tissue until no more chunks are visible. Transfer 1ml of the liquid in to a microfuge tube, placed it on ice. Spin sample at top speed in the microfuge. Transfer the liquid supernatant in to second microfuge tube. Store sample in -80^0c.=

SDS – PAGE [sodium dodecyl sulphate – poly acryl amide gel electrophoresis]

Thoroughly clean and dry the glass plates and spaces, then assembles them properly. Hold the assembly together with bulldog clips. Clamp in an

upright position. 2% agar [melted in boiling water bath] is then applied around the edge of the spacers to hold them in place and seal the chamber between the glass plates. Prepare a sufficient volume of separating gel mixture. Mix gently and carefully, pour the gel solution in the chamber between the glass plates. A layer distilled water on top of the gel and leave to set for 30 -60 min. Prepare stalking gel. Remove the water from the top of the gel and wash with a little stalking gel solution. Pour the stalking gel mixture, place the comb in the stalking gel and allow the gel to set [30- 60 min]. After the stalking gel has polymerized, remove the comb without distorting the shape of the well. Carefully install the gel after removing the clips, agar etc. in the electrophoresis apparatus. Ill it with electrode buffer and remove any trapped air bubble at the bottom of the gel. Connect the cathode at the top and turn on the DC- power briefly to check the electrical circuit. The electrode buffer and the plates can be kept cooled using suitable facility so that heat generated during the run is dissipated and does not affect the gel and resolution. Cool the sample solution and take up 50µl of sample in micropipette and carefully inject it in to sample well through the electrode buffer. Marking the position of the wells on the glass plates with a marker pen and the presence of coo massive brilliant blue R-250 in the sample buffer facilitate easy loading of the sample. Similarly, load a few wells with standard marker protein in the sample buffer. Turn on the current to 10 – 15mA for initial 10 – 15 min until the sample travel through the stalking gel. Stalking gel helps the concentration of the sample then continue the run at 30mA until the coo massive brilliant blue reaches the bottom of the gel [about 3hrs]. However, the gel may be run at high current [60-70mA] for short period [1h] with proper cooling. After the run is complete, carefully remove the gel from between the plates and immerse in staining solution for at least 3hrs or over night with uniform shaking. The protein absorbs the coo massive brilliant blue Transfer the gel to suitable container with at least 200 – 300 ml distaining solution and shake gently and continuously. Dye that is not bound to proteins is thus removed. Change the destainer frequently, particularly during initial periods, until the back ground of the gel is colorless. The proteins fractionated in to bands are seeing colored blue. The gel can be photographed or stored. (Sadasivam S et al., 1996)

ANTIMICROBIAL ACTIVITY OF GUAVA LEAF EXTRACT

EXTRACTION METHOD USED ON GUAVA

The leaf samples were washed in tap water, dried, and placed into a blender to be grounded into powder. 99.5%ethanol and distilled water were used as solvents, and it was used for the maceration extraction procedure. The leaf powder was added to the solvents to make a 20% concentration. The mixture has made in sterile 100 mL Erlenmeyer flask wrapped in aluminum foil to

PROTEIN PROFILE FOR GUAVA PEROXIDASE ENZYME

avoid evaporation and exposure to light for 3 days at room temperature. The flasks were placed on a platform shaker at 70 rpm. After 3 days of soaking in solvent, the mixtures were transferred to 50 mL tube and centrifuged for 10 min at 4,000 rpm at 25°C. The supernatant was collected and stored at 4°C until use.

ANTIMICROBIAL ACTIVITY OF GUAVA LEAF EXTRACT

Antimicrobial susceptibility testing was done using the well-diffusion method. The plant extract was tested on Mueller Hinton agar plates to detect the presence of antibacterial activity. All plates were inoculated with the test bacteria's such as E.coli and S.aureus. Uniform streaking has done with a sterile "L"rod. The plates are allowed 3 to 5 min to dry the excess moisture.5mm diameter wells were punched in to the medium using a sterile borer. Different concentrations (20µL, 40µL, 60µL, 80µL) of the test extracts were dispensed into each well after the inoculation of the plates with bacteria. The same extracts were used on each plate, with a total of three plates. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The plates are sealed with petriplate wrapper, labeled, and placed in an incubator set to 37°C. After 24 hours of incubation, each plate was examined for inhibition zones. A ruler was used to measure the inhibition zones in millimeters. Every experiment was carried out in parallel, and the results represented the average of at least three independent experiments. (Bipul Biswas et al., 2013)

RESULTS AND DISCUSSION

The yield percentage of plant extract was found to be 22.4%.25grams of fresh Guava leaves were used for the extraction procedure. Phosphate buffer was used as solvent. The yield percentage of leaf extract was used for further studies.

DETERMINATION OF GUAVA PEROXIDASE ENZYME

The yield percentage of plant extract was found to be 22.4%. 25grams of fresh Guava leaves were used for the extraction procedure. Phosphate buffer was used as solvent. The yield percentage of leaf extract was used for further studies.

Peroxidase activity was determined calorimetrically by using citrate phosphate buffer containing giaiacol and hydrogen peroxidase.The optimum density of the enzyme was found to be 0.23nm.The enzyme activity obtained was 0.24µ/ml.(Table 3) Enzyme activity was found to be = 0.246u/ml

Peroxidase activity was determined calorimetrically by using citrate phosphate buffer containing giaiacol and hydrogen peroxidase.The optimum density of the enzyme was found to be 0.23nm.The enzyme activity

obtained was 0.24µ/ml.

DETRMINATION OF SPECIFIC ACTIVITY OF GUAVA PEROXIDASE BY BRADFORD METHOD (Table3.1.1) (Fig 3)

1ml of unknown contains 7µg of the protein

The given sample contain 7.0mg of protein

The specific activity of the gouva peroxidase was found to be 0.003 u/ml/mg of protein

THERMAL INACTIVATON OF GUAVA PEROXIDASE ENZYME

The optimum density of the sample was found to be T_1 - 0.01, T2 - 0.02, T3 - 0.03, T4 - 0.06. T5 _ 0.08. T_5 was showing the maximum activity among all the tests. The activity of Guava peroxidase was stable at three different time intervals (5min, 10min and 15min).The activity of the enzyme was found to be 0.09U/ml.The Guava peroxidase has a uniquely high thermo stability .The thermal inactivation was studied in 10mM Tris – Hcl buffer pH (8.0). The activity of Guava peroxidase was found stable in 3 different time intervals such as 5min, 10min, and 15min where as the activity was differing in rest of the time intervals.(Table 4 and Fig 4)

OPTIMIZATIZATION

EFFECT OF PH ON GUAVA PEROXIDASE ENZYME

The maximum activity of Guava peroxidase was found to be 0.65U/ml at pH 5.

Guava peroxidase showed the maximum activity at pH 5 and it get decreased when pH increases (Dubey et al, 2000).One unit of Guava peroxidase activity is defined as the amount of enzyme which catalyses the conversion of one micromole of hydrogen peroxide per minute at 25^{0}c. The graph is obtained by plotting pH Vs Guava peroxidase enzyme activity.(Table5andFig5)

EFFECT OF TEMPERATURE ON GUAVA PEROXIDASE ENZYME (Table 5,Fig 6)

The maximum activity of the Guava peroxidase was found to be 0.06U/ml at 70^{o}c

The effect of temperature on Guava peroxidase was studied at different temperature by using acetate buffer (5.0), guaiacol as indicator and hydrogen peroxide as substrate. It started decreasing while moving towards high temperature. The activity of Guava peroxidase was showing slight increase from 4^{0}c to 37^{0}c. There was a sudden increase of enzyme activity at 70^{0}c. The activity of the enzyme was seems to be 0.06 at 70^{0}c. Guava peroxidase showed maximum activity at 70^{0}c.

One unit of peroxidase activity is defined as the amount of enzyme which catalyses the conversion of one micromole of hydrogen peroxide per minute at 25^{0}c

EFFECT OF SUBSTRATE CONCENTRATION ON GUAVA PEROXIDASE ENZYME

The maximum activity of the Guava peroxidase was found to be 75U/ml

Effect of hydrogen peroxide concentration on peroxidase activity was studied using the reaction system consisted of 1ml of enzyme extract , 1ml of guaiacol, respective volume of hydrogen peroxide(1- 5 ml) and acetate buffer pH(5.0). In each measurement, the final volume of the reaction solution was changed based on the volume of the substrate taken in the tubes. The activity of the Guava peroxidase enzyme was more when the concentration of the substrate was increased. The maximum activity of the Guava peroxidase was seen in the test containing 5ml of substrate concentration. (Table 5.3.1: Figure 7)

PROTEIN PROFILE FOR GUAVA PEROXIDASE ENZYME

SDS-PAGE analysis of Guava peroxidase was elecrophorized. The protein peroxidase has travelled a distance about 3.5cm from the well. The protein peroxidase migrated in SDS– electrophoresis as a single band corresponding to the molecular weight of 59KDa. (Fig 8, Fig 9 and Table 6)

ANTIMICROBIAL ACTIVITY OF GUAVA LEAF EXTRACT

The results indicate that the solvent, ethanol is better than distilled water for the extraction of anti microbial activity of *Psidium guajava* leaf. The results also indicate that the plant extracts have no antibacterial effect on the Gram-negative bacteria, showing that they do not contain active ingredients against the organism. The observed inhibition of gram positive bacteria*, s.aureus* suggests that Guava possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using ethanol as the solvent. On the basis of the present finding, *P. guajava* leaves possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and diseases caused by *S. aureus* .Different concentrations of the ethanolic and distilled water extracts of the leaves of psidium Gujava were used to perform antimicrobial activity .The ethanolic extract showed antimicrobial activity against the isolate of S.aureus and there was no antimicrobial activity when distilled water was used as solvent. The plate containing the isolate *S.aureus* with 80µl of ethanolic extract of *Psidium guajava* was showing the antimicrobial activity with significant zone of inhibition. (Fig 10,11,12 and Table 7)

Conclusion

In the present study, was aimed to extract the peroxidase enzyme from the leaves of Psidium guajava plant and optimize its activity at different temperatures,

Table 5.1.1: The activity of guava peroxidase on different pH

pH	Activity of peroxidase enzyme(U/ml)
3.6	0.10
4.2	0.15
5.0	0.65
5.5	0.45
6.0	0.25
6.5	0.25
7.5	0.25
8.0	0.50

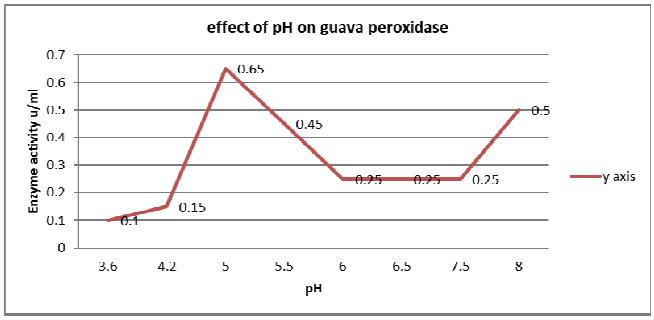


Figure 5: The effect of pH on Guava peroxidase enzyme

EFFECT OF TEMPERATURE ON GUAVA PEROXIDASE ENZYME

Table 5.2: Effect of temperature on Guava peroxidase enzymeTable

Number of tubes	Volume of phosphate buffer (ml)	Volume enzyme (ml)	Volume of guaiacol (ml)	Volume of H_2O_2(ml)	Incubation temperature		OD at 450
1	1ml	1ml	1ml	1ml	4^0	Incubate the tubes at respective temperature for 2 min	0.02
2	1ml	1ml	1ml	1ml	15^0		0.07
3	1ml	1ml	1ml	1ml	37^0		0.11
4	1ml	1ml	1ml	1ml	50^0		0.20
5	1ml	1ml	1ml	1ml	70^0		0.14

Table 5.2.1: The activity of guava peroxidase on different temperatures

Temperature(°c)	Activity of peroxidase enzyme (U/ml)
4^0	0.01
15^0	0.02
37^0	0.03
50^0	0.04
70^0	0.06

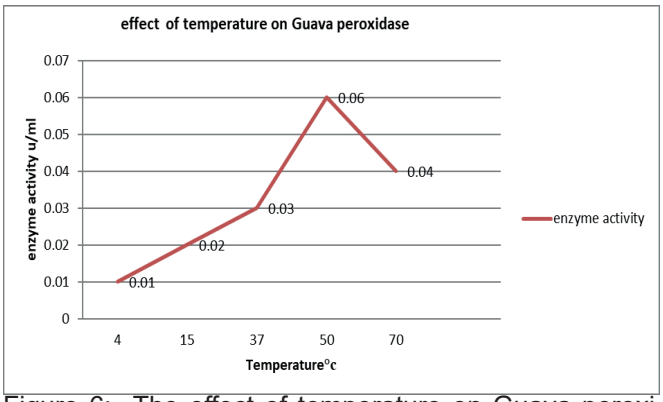


Figure 6: The effect of temperature on Guava peroxidase

EFFECT OF SUBSTRATE CONCENTRATION ON GUAVA PEROXIDASE ENZYME

Table 5.3: Effect of substrate concentration on guava peroxidase enzyme

Sl.no	Volume of buffer (ml)	Volume of enzyme (ml)	Volume of guaiacol (ml)	Volume of hydrogen peroxide (ml)	OD at 450 for every 15sec up to 3 min						
					15 (s)	30 (s)	45 (s)	60 (s)	1m	2m	3m
1	1ml	1ml	1ml	1ml	.32	.32	.32	.32	.33	.34	.35
2	1ml	1ml	1ml	2ml	.24	.24	.24	.24	.25	.26	.26
3	1ml	1ml	1ml	3ml	.21	.21	.21	.22	.23	.23	.24
4	1ml	1ml	1ml	4ml	.13	.12	.12	.12	.12	.11	.10
5	1ml	1ml	1ml	5ml	.17	.16	.15	.15	.16	.17	.18

Table 5.3.1: Activity of guava peroxidase on different substrate concentration

Volume of substrate(ml)	Activity of perroxidase enzyme(U/ml)
1ml	20
2ml	30
3ml	50
4ml	65
5ml	75

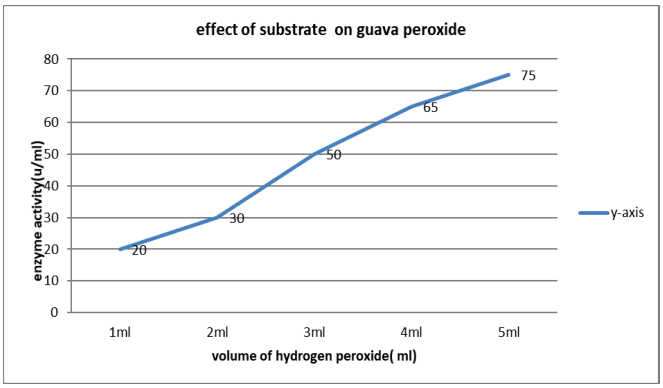


Figure 7: The effect of substrate concentration on Guava peroxidase enzyme

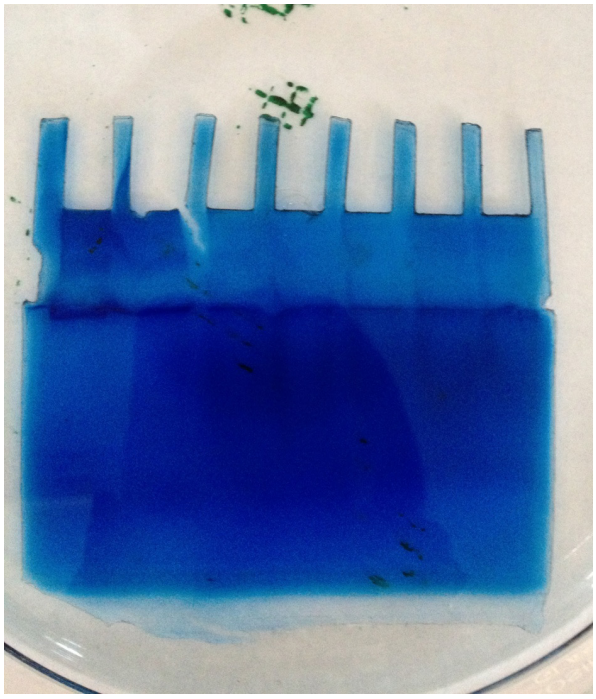


Figure 8: SDS-PAGE analysi

Table 6: Determination of molecular weight of the sample

Molecular weight of the marker (KDa)	Distance travelled from well (cm)
97	1.5
64	3
51	4.5
39	6.6
28	9
19	11.6
14	14

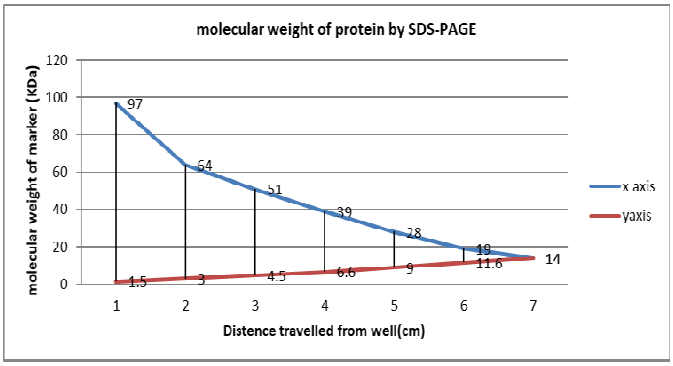


Table 6: Determination of molecular weight of the sample

pH and substrate concentration. The leaves of psidium guajava were collected from the village area. The extract of the psidium gujava leaves were prepared and which is used for the following studies. The activity of the Guava peroxidase was determined by enzyme assay and the specific activity of the enzyme was determined by protein estimation. The amount of the protein present in the Guava sample was found to be 7.0mg.The specific activity of the protein was 0.03u/ml/mg.

The study was also focused on the thermo stability of the Guava peroxidase enzyme. Thermal inactivation was studied in 10mM Tris – Hcl buffer pH (8.0). The activity of Guava peroxidase was found stable in 3 different time intervals. The activity was seemed to be stable at time intervals 5, 10, and 15min.The optimization of Guava

ANTIMICROBIAL ACTIVITY OF GUAVA LEAF EXTRACT

Figure10: Sample showing no zone of inhibition against E.coli

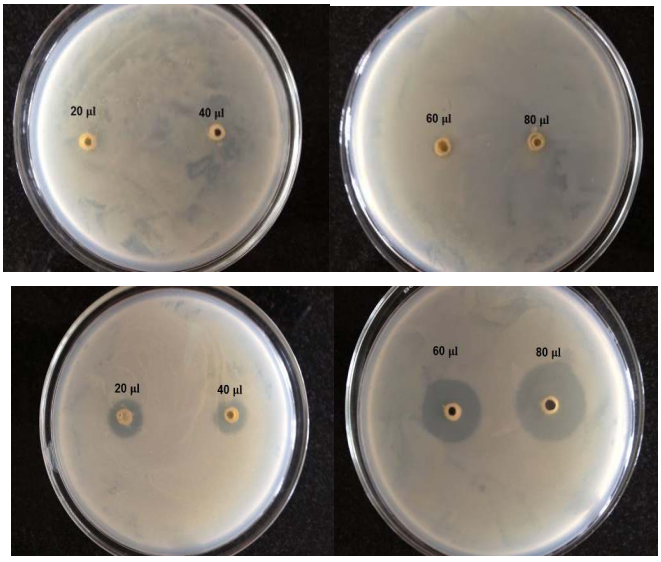


Figure 11: Sample showing zone of inhibition against S.aureus

Figure 12: Control with acetone and water against S.aureus

Table 7: Determination of antibacterial activity

Plant extract	Concentration of the extract	Zone of inhibition(mm)	
		S.aureus	E.coli
Ethanol (solvent)	20µl	10mm	-
	40µl	12mm	_
	60µl	18mm	-
	80µl	22mm	_
Distilled water (solvent)	20-80µl	_	_

peroxidase and its protein profiling by SDS-PAGE were also done to find out the activity of the particular enzyme at different pH, temperature substrate concentration, purity and the molecular weight. The molecular weight of the protein present in the Guava sample was 55KDa. The maximum activity of the Guava leaf peroxidase was on pH 5.0, temperature 70ºc and substrate concentration 5ml.

The present study demonstrates the antimicrobial potential of *Psidium guajava* leaves extract by using various solvents. The results indicate that the solvent, ethanol is better than distilled water for the extraction of antimicrobial activity of *Psidium juajava* leaf. The results also indicate that the plant extracts have no antibacterial effect on the Gram-negative bacteria, showing that they do not contain active ingredients against the organism. The observed inhibition of gram positive bacteria, *s.aureus* ssuggest that Guava possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using ethanol as the solvent. On the basis of the present finding*, P. guajava* leaves possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and/or diseases caused by *S. aureus*.

References

1. Smitha Jain, Morna-Ghons A,Fevereiro PS;2010. plant as a source of medicinal agents. International Medicinal Research Journal. 66(2): 924-931.
2. Nair, arnok.P, Chantai.2004.Traditional medicinal properties of plants.55 (2):385-329.
3. Cox and Blick.1994 .Identification of secondary metabolites and its role in different plant source.17:583-592.
4. Rasool Hssun.2012.Nutritional properties of plants. International plant biotech Reserch Journel., 66(8):45-78.
5. Royale J.2008.Phytochemical investigation of medicinal plants 5; 954-987.
6. Sing J and Joy.G.K.2008.Determination of structural properties of Horse radish peroxidase.12 (9):324-336
7. Ghaemmaghami K. 2010. Some novel applications of peroxidases. International Journal of enzyme.44 (6):1 56-179
8. Rao et al., 2007.Studies on Anylitical application of peroxidase enzyme .44(4).234-256
9. Robert E, 1992.Purification of peroxidase from Psidium gujava.International journal of botany. 77(8)234-289.
10. Jennifer,1994.Antimicrobial activity of Riccinus communis.International Journal of Microbiology. 88(6)298-345).
11. Bullen.A and Kulezca..2007.peroxidase activity on Cabbage leaf. International Journal of Biochemistry.66 (6):123-145.
12. Kumare.J.J.2000.Idntification of peroxidase enzyme activity from raddish.33(6)134-255.
13. Sakhrov and Bipul Bisvasa.2013.Antimicrobial activity of guava leaf extract. Journal Of Microbiology.33 (9):453-460.
14. Meighy Necke..2000.Antimicrobial activity of Osamum sandom.journal of Microbiology. 55(8):233-248.
15. Sameer and sivan.2001.Thermostability of oil pam peroxidase.Resistance .Journal of biochemistry. 77(7):123-23.
16. kamala.M..2000.Extraction and Isolation of peroxidase enzyme Soyabean.International Journal of Biotechnology. 88(8):347-358.
17. Galeavatol.YU.2000.Extraction and isolation of peroxides from the leaves of onion.234-256.
18. Sadasivam.S and Manikam.A.1966.Laboratory Manual.123-300.
19. Castillo.L et,al.2000.Thermal inactivation of Soyabean peroxidase.Journal of Biochemistry. 55(5):45-32.
20. Jayaram.K ,Prakash P,and Bikash.M.2012. Evaluvation of Antimicrobial activitity of Aloe vera .15(3)246-248.
21. Aires barose.H 1999 .Invitro antimicrobial activity of Mangnifera indica. (2012).234-238.
22. Morna.H and Serralherio.(1999). Application of factorial design to the optimization of peroxidase activity biotechnology.55(9):234-239.
23. Rungaviriyachai P, Arnok D.2010.Optimization and determination of peroxidase activities in hot pepper. International food research journal.17:385-392.
24. Nelson and Robert E. 1992.Peroxidase activity in the leaf elongation Zone of fescue.55(9):34-39.
25. Suha, O. A,Babiker, E. M. and Babiker, E E.2000. Thermostability at different pH.
26. Niyang Thalong.2001.Levels of peroxidase extracted from four vegetablesAgri Food Chemistry journal. 55(7):121-140
27. L. Sergio.2007 Thermostability of Peroxidases from Artichoke, Food Technol Biotechnology. 45 (4) 367–378.
28. Bania and Rita Mahanta .2000.Evaluvation of peroxidase from varios vegetable sources.55 (8):12-19.
29. Clemente.E.1998 purification and thermostability

of isoperoxidase from orange.Journal of biochemistry.49:29-36.

30. Dunford .H B.1991.Structural properties of Horseradish .22(9):35-50.
31. Gazearyan and Lagrinini .L N .1996.Purification and unusual kinetic properties of Tobacco anionic peroxidase.Journal of phytochemistry.41 (8):322-328.
32. Deepa.S.S and Armugan.C.2000.Purification and charecterisation of soluble peroxidase from oil palm. phytochemistry.61(3):503.
33. Esnault.R and s.hibbar.R.N.1997.Peroxidases and plant defense.newslett. 11: 27-34.
34. Kamal A.J.K and Beher.D.V.2002.Thermal and conformational stability of seed coat soyabean peroxidase.biochemistry.41:034-9042.
35. Davis .D.M,Jhons .P.1976.the kinetics of formation of horse raddish peroxidase biochem journal.157(1):247-253.
36. Colona.S,Gaggero, Richelmic P.P.1999.recent biotechnological developments in the use of peroxidase .trends biotechnology. 17:163-168
37. Chmeilinicka.J.1966.studies on cabbage peroxidase .effect of pH and temperature on enzyme activity .23:461-476
38. Veitch.L.C .2004.horseraddish peroxidase:a modern view of classic enzyme. phytochemistry. 65:249-259
39. Doneford H.V.1991.Horseraddish peroxidase: structure and kinetic properties. peroxidase in chemistry and biology.15(3):121-130.
40. Lebedeva.o.v, Ugaroa N.N 1977.Kinetic study of oxidation by hydrogen peroxide in the presence of horseradish peroxidase. biokhimia., 42 (8):2-1379.
41. Endreva.A,Karthijeeva R.1997.Involvment of peroxidase in heat shock response of Bean plant. plant perox.newslet.11:27-35.
42. Pathmaraja N.A,Anandhanarayan S.2009. Antimicrobial activities of Camera lucida. international journal of microbiology.89:411-420.
43. Klibenow A.M.2000.Antifungal activity of osimum santum.journal of microbiology 41:034-090.
44. Dolmen D.1975.Antibacterial activity of Euphobia hirita.journel of botany and microbiology. 17:163-168
45. Guriya,R,Mentonekka.2000.Extraction and isolation of peroxidase from sun flower. international journel of plant biotechnology.55(6):272-292
46. Kermasha S and Metakhe M.1988.Studies on apple seed peroxidase.journal of food chemistry .,55:247-256
47. Poppa G,Ollia B.2009.Peroxidase activity in papaya plants transformed by *Agrobacterium agizogenus. journal of plant biotechnology., 54:41-46*
48. Eriza jeisy and Yualsu Galave.2000.Purification and stability of peroxidase of African oil palm.9(1)125-132