CHAPTER 2

LITERATURE REVIEW
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2.1 Local names, Taxonomy of A. paeoniifolius

English (Elephant foot yam, Whitespot Giant Arum, Sweet Yam, Telinga Potato); Hindi name(Suranakanda, Zimikanda); Bengali name(Ole); Fijian(Suran); Japanese( Koniaku, Konjac, Konnyaku);Thai(Buk Khang);Tamil (kizhangu); Kannada (suvarna gedde);Oriya (ulo); telugu( kanda gadda).

Scientific Classification

Kingdom: Plantae
Division: Angiosperms
Class: Monocots
Order: Alismatales
Family: Araceae
Genus: Amorphophallus
Species: paeoniifolius
Synonyms: A campanulatus

Figure 2.1: Photograph of A. paeoniifolius (a: whole plant, b: corm)
2.2 Ayurvedic properties

*Rasa- Katu, Kashaya*

*Guna-Ruksha, Tikshna, Guru, Vishada, Laghu*

*Vipaka-Katu*

*Veerya- Ushna*

*Karma- external-Shothahara, Vedanasthapana Internal-Arshaghna, Vatahara, Kaphahara, Yakrit-Uttejaka.*

2.3 Botanical description

*Amorphophallus* is a perennial, terrestrial underground hemispherical depressed dark brown corm of approximately 20-25 cm in diameter which bears flowers and fruits in the month of April – May. It bears leaves that are solitary which are 30-90 cm broad; Inflorescence consist of a foliar organ, the spathe, which usually envelops a stalk –like organ, the spadix. The flowers are tiny, monoecious, strongly reduced and are found at the base of the spadix. Raphides of the *Amorphophallus campanulatus Blume* (syn. *paeonifolius*) isolated from tuber are pointed at one end and, square at other end, cross section is ‘X’-shaped at pointed end and they are asymmetrical[9].

2.4 Propagation, cultivation and storage

Hot and humid climate provides better growth of Elephant foot yam. Humid climate supports in the initial stages of crop growth where as dry climate facilitate tuber bulking. Well-distributed rainfall of 1000-1500 mm is helpful for both crop growth and high tuber yield. Well-drained, fertile, sandy-loam, black soil is best for elephant foot yam cultivation. Elephant foot yam is a long duration crop and generally matures in 6-7 months. Crops can be harvested at different stages of development initially from 6-7 months of plantation upto 4 years whenever required. The crop is cultivated as a mixed crop in the fields of banana, ginger and groundnut. Cut tuber pieces are used as planting material and take 3- 4 weeks time for the development of sprouts. Vegetative propagation carried out through corms planted in the prepared pits (40cm x 40 cm x 40 cm), filled with decomposed cow dung and sandy loam soil [10]. Sprouting starts after 2-3 months of tuber storage. Tubers are stored in ventilated,
shaded and cool place in single layer or in two–three layers with monitoring and removal of infected tubers, for avoiding its heavy rotting.

2.5 Traditional uses of corms

The tubers are a delicacy in food and rich in nutrients is much popular as a vegetable in various delicious cuisines. The tuberous roots of the plant posses blood purifier properties and have been used traditionally for the treatment of piles, abdominal disorders, tumours, enlargement of spleen, asthma and rheumatism[11]. They are traditionally used in arthralgia, elephantiasis, tumors, inflammations, hemorrhoids, hemorrhages, vomiting, cough, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic, constipation, helminthiasis, hepatopathy, spleenopathy, amenorrhea, dysmenorrhea, seminal weakness, fatigue, anemia and general debility. The tuberous roots of the plant have also been reported to possess tonic, stomachic and appetizer properties [11]

2.6 Major nutrient and chemical constituents of Amorphophallus corm

Amorphophallus is a good source of energy, sugar, starch, proteins as well as minerals. Average nutritional profile contains Starch (11-28%), Sugar(0.7-1.7%), Protein(0.8-2.60%), Fat(0.7-0.40%), mean energy value(236-566.70KJ/100g). The most abundant macro mineral is potassium (327.83 mg/100 g), phosphorus (166.91 mg/100 g), calcium (161.08 mg/100 g) and iron (3.43 mg/100 g). Range of macro minerals and soluble oxalate between different varieties; K (230-417mg/100g), P (120-247 mg/100g), Ca (131-247 mg/100g), Fe (1.97-5.56 mg/100g), Mn (0.19-.65mg/100 g), Zn(0.12-1.92 mg/100 g), Soluble oxalate (6.65-18.50 mg/100g. The mean soluble oxalate content (13.53 mg/100g) was safe from the viewpoint of accumulation of urinary oxalate leading to kidney stones [12].

2.7 Phyto-chemical screening

Qualitative assay of different solvent extracts of Amorphophallus paeoniifolius was carried out for the presence of phytoconstituents. Petroleum ether, Methanol, Chloroform and water extracts were tested further tested for the presence of different phytoconstituents. The petroleum ether extract contain alkaloids, steroids, fats & fixed oil. The chloroform extract contain alkaloids. The plant methanol extract contains alkaloids, steroids, flavonoids and carbohydrates. The aqueous extract contains flavonoids, tannins, proteins and carbohydrates. Ethyl acetate, hexane extract contains alkaloid, flavones, carbohydrate and saponins.
Methanolic extract (ME), 70% Hydro-alcoholic extract (AE) of "Amorphophallus paeoniifolius" was analysed for flavonoidal content (FC) in terms of Rutin and Total Phenolic Content (TPC) was measured in terms of catechol equivalent. Thin Layer Chromatography (TLC) study of methanolic extract was conducted. The Flavonoidal content of ME and AE were found to be 46.33 mg/g and 36.88 mg/g respectively [13-14].

2.8 MEDICINAL USES OF CORM

2.8.1 Gastro protective ability

Free radical species play important role in gastrointestinal ulcerogenesis. Phytochemical constituents has showed the presence of polyphenols, which also posses anti ulcer activity. Methanolic extract of Amorphophallus has the gastro protective ability against pylorus ligation induced gastotoxicity in albino rats. Methanol extracts of the corm has shown the increased GSH levels and inhibition of lipid peroxidation in dose dependent manner i.e. 250 and 500 mg/kg. Treatment with methanol extracts showed reduction in gastric volume, free acidity, total acidity, pH and ulcer score .The protection percentage in dose dependent manner was, 250 mg/kg showed 67% and 500 mg/kg showed 85.5% activity respectively in comparison with standard Lansoprazole 80.50%

2.8.2 Analgesic activity

Oral dose of 250 and 500 mg/kg of methanol extract, showed significant analgesic activity in mice. The methanol extract suppressed dose dependently the frequency of acetic acid-induced writhing in mice[16]. Analgesic activity was also confirmed by tail flick method and acetic acid induced writhing response method by using diclofenac sodium as standard. The intraperitoneal administration of methanol extract of Amorphophallus paeoniifolius tubers (250, 500 mg/kg) induced a significant analgesic activity in a dose-dependent manner[17].

2.8.3 Anticonvulsant Activity

Petroleum ether extracts of Amorphophallus paeoniifolius at the dose of 200, 300, 400 mg/kg were used for the effects on the onset of convulsion in Isoniazid (INH) induced mice model. Diazepam at the dose of 4 mg/kg was used as the standard drug. The group pre-treated with standard diazepam had late onset of convulsion. Petroleum ether extract of Amorphophallus paeoniifolius in doses of 200, 300, 400 mg/kg significantly increased the latency of onset of
convulsions. Petroleum ether extracts showed dose-dependent activity regarding onset of convulsion[18].

2.8.4 Antihelmintic activity

The methanolic extract of the tubers showed antihelmintic activity against *Pheretima posthuma* and *Tubifex tubifex*. The extract with the concentrations of 25, 50 and 100 mg/ml were tested in the bioassay, which involved determination time of paralysis time and death time of the worms. Methanolic extract showed dose dependent antihelmintic activity as compared to a standard drug piperazine citrate. The extract exhibited significant antihelmintic activity at highest concentration of 100 mg/ml. Piperazine citrate (10 mg/ml) was included as standard reference and distilled water as control. The extract was found not only to paralyze (Vermifuge) but also to kill earthworms (Vermicidal)[19].

2.8.5 Antidiarrhoeal activity

Swiss Albino rats of either sex weighing 150-180 g was used for study and anti-diarrhoeal activity was evaluated by castor oil-induced Diarrhoea model. The ethanolic extract of the *A. paeoniifolius* leaves, (doses: 100, 200 and 400 mg/kg), reduced the total number of faeces as well as diarrhoeic faeces in a dose dependent manner and the results were statistically significant (p< 0.05). Ethanolic extract of *A. paeoniifolius* leaves exhibited a statistically significant reduction in the severity and frequency of diarrhoea produced by castor oil, which indicates wide range of usefulness of this plant in secretary and functional diarrhoeas [20].

2.8.6 Anti-inflammatory activity

Anti inflammatory activity was observed by using carrageenan induced paw edema model in rats. Among the petroleum ether, chloroform, methanol and water extracts, the methanol extract of *Amorphophallus paeoniifolius* displayed prominent anti-inflammatory activity while the chloroform extract had milder activity. Diclofenac sodium at the dose of 5 and 10 mg/ kg was used as standard drug. 3 hours after carrageenan injection, the methanol extract a dose of 200 and 400 mg/kg, produced 37.5% and 45.83% inhibition in swelling index as compared to control group. The control group where the maximum swelling index reaches up to 59.2 at 5th hour where as in case of groups received diclofenac sodium 10 mg/kg and methanol extract 400 mg/kg the maximum swelling index were 16.02 and 23.29 at 2nd and 3rd hour respectively[21].
Methanol extracts of 200mg/kg and 400mg/kg with standard drug aspirin at the dose of 100 mg/kg also found to produce Anti-inflammatory activity. The effect of *Amorphophallus campanulatus* tuber extracts was dose as well as time dependent, both extracts shows maximum inhibition at 180 min [(200 mg/kg) 48.44 % and (400 mg /kg)60.35%] [22].

2.8.7 Antibacterial activity

Aqueous and organic solvent extracts of *Amorphophallus* were evaluated for its antibacterial potential by using disc diffusion method, against pathogenic strains of gram-positive bacteria (*Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Streptococcus β-haemolyticus*) and gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris*, *Shigella dysenteriae*, *Shigella sonnei*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Salmonella typhi*)[23-24]. Antibacterial activity of these chloroform and petroleum ether extract have found by flavonoid and triterpenoid compound, 3,5- diacetyltambulin, ambylone respectively [25] [23]. Different extracts differed significantly in their anti-bacterial properties with the benzene extract being very effective followed by petroleum ether, chloroform and ethyl acetate extracts. Aqueous and methanol extract showed very least activity [24]. Petroleum ether, chloroform and benzene extract has established good values on antibacterial activity against both gram-positive and gram-negative bacteria compared to the other extracts.

2.8.8 Antioxidant Activity

Antioxidant has a characteristic ability to trap free radicals. *Amorphophallus* species contains some kind of phytochemicals like polyphenols and flavonoid with antioxidative effect. Hexane extract and methanolic extract of *A. campanulatus* tuber were evaluated for phytochemical screening and in vitro antioxidant activities using DPPH, hydroxyl radical, reducing power and total antioxidant capacity assays. The total phenolic and flavonoid contents were also investigated. The protective potential of two different doses of methanolic extract (125 and 250 mg/kg) was also evaluated against thioacetamide (TAA) induced oxidative stress in rats. Silymarin was used as a standard drug control. In vitro studies revealed that methanolic extract has higher antioxidant and radical scavenging activity than hexane extract, which may be attributed to its higher phenolic and flavonoid content. ACME significantly prevented the elevation of serum AST, ALT, ALP, LDH, and tissue malondialdehyde levels (P < 0.05). Hepatic and renal GSH, GST, GR, GPx, and catalase levels were remarkably increased by the treatment with the extract. Quantification of
Histopathological changes also supported the dose dependent protective effects of metahonic extract [26].

Antioxidant activity and radical scavenging potential of ethanolic extracts of *Amorphophallus paeonifolius* was studied for the inhibition of lipid peroxidation estimated in terms of thiobarbituric acid reactive substances (TBARS) and the levels were reduced by 4.3% to 67.2% in a dose-dependent manner. Methanolic extract was analyzed for scavenging capacities based on DPPH assay (1, 1-diphenyl-2-picrylhydrazyl-2-radical) and percentage inhibition activity based on 2, 2-azinobis-(3-ethyl) benzo-thiozoline-6-sulfonate (ABTS+) and H$_2$O$_2$. The *A. paeonifolius* extract showed a maximum of 68.6% of DPPH scavenging activity and the maximum inhibition of 74% and 67.2% in the case of ABTS and H$_2$O$_2$, respectively. The antioxidant efficiency and inhibition of oxidation of the extract was found to be dose-dependent at the tested concentrations of 1-50 micro g/ml. High-performance thin layer liquid chromatography (HPTLC) profile of the extract suggests the presence of polyphenols such as gallic acid, resveratrol, quercetin and two unidentified compounds. The results suggest that the ethanol extract of *A. paeonifolius* has a potent antioxidant activity in vitro and can be utilized as an effective and safe source of antioxidant [27].

### 2.8.9 Anti Tumour Activity

The ethanolic extract of *Amorphophallus paeonifolius* has shown significant antitumor and antioxidant effect in animals. Effect of ethanolic extract on RBC, WBC, Hb & Neutrophils found as a-P< 0.001, b-P<0.01, c-P<0.05, ns-non significant, Effect of AP-extract on tumor latency and tumour burden were found as extremely significant at P<0.001. The present preliminary investigation suggests that *Amorphophallus paeonifolius* tuber stimulate both cellular and humoral immunity[28].

### 2.8.10 CNS depressant activity

It was found that petroleum ether extract at doses of 100, 300 and 1000 mg/kg showed significant CNS depressants activity in mice. The intra-peritoneal administration of petroleum ether extract of *Amorphophallus paeonifolius* tubers (100, 300, 1000 mg/kg) induced a significant decrease in locomotor activity and grip test in a dose-dependent manner. The percentage decrease in locomotor activity are 16.53 (P>0.05), 56.77 (P<0.01), 73.36 (P<0.01) (n=6) and percentage decrease in activity in grip test are 10.38 (P>0.05), 62.67 (P<0.01) and 70.78 (P<0.01) (n=6) after 1 hour the intra-peritoneal administration of
Amorphophallus paeoniifolius at the doses of 100, 300, 1000 mg/kg respectively[29]. A significant synergistic effect of the petroleum ether extract of Amorphophallus paeoniifolius was seen with diazepam as compared to that of phenobarbitone [30]. Effective dose (ED$_{50}$) for petroleum ether extract for the CNS depressant activity was calculated to be 250 mg/kg. Both of the phenobarbitone and diazepam exert their CNS depressant effect by acting on the GABA receptor. So it was concluded that the components present in the petroleum ether extract may bind with the α subunit and facilitate the GABA mediated Cl- channel opening, thus hyperpolarizing the cell and show CNS depressant action. Diazepam is a benzodiazepine receptor agonist it was concluded that extract has agonistic activity with benzodiazepine receptor, which might similar to that of diazepam. Further investigations need to be done to understanding of molecular mechanism of action and signal transduction of the components present in petroleum ether extract of A. paeoniifolius regarding CNS depressant activity.

2.8.11 Hepatoprotective activity

Amorphophallus paeoniifolius showed hepatoprotective activity against paracetamol and carbon tetrachloride induced liver damage in rats. The methanol and aqueous extract against paracetamol and ethyl acetate extract, aqueous and ethanolic extracts against carbon tetrachloride showed comparable reduction in the elevated levels of some serum biochemical indicators like serum glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT) and serum alkaline phosphatase (sALP), serum bilirubin (SB) similar to silymarin and Liv-52 as standard hepatoprotective agents [31-33].

2.8.12 Immunomodulatory Activity

Methanol extract of Amorphophallus campanulatus showed immunomodulatory activity under normal and cyclophosphamide induced immunosuppressive conditions in Swiss albino mice models [34]. Tuber orally at doses of 250 and 500mg/kg was investigated for immunomodulatory activity using charcoal clearance, spleen index and delayed-type hypersensitivity (DTH) response models. The extract exhibited immunomodulatory activity by causing a significant decrease in charcoal clearance, delayed-type hypersensitivity (DTH) response and spleen index[35]. The methanol extract of Amorphophallus campanulatus tuber significantly and dose dependently suppressed the immune system in mice because of fatty acids and phytosterols in the extract.
2.8.13 Cytotoxic and apoptotic activities

The methanolic extract and chloroform extract of *Amorphophallus campanulatus* found to produce considerable cytotoxicity and induce apoptosis in human liver cancer cell line, PLC/PRF/5 and colon cancer cell line, HCT-15[36-37]. These extracts have potent cytotoxic and apoptotic activity and thus it could be explored as a novel target for anticancer drug development. Furthermore, the sub fractions of methanolic extract dose-dependently suppress the proliferation of HCT-15 cells and PLC/PRF/5 cells by inducing apoptosis.

2.9 INSECTICIDAL POTENTIALITY

*Amorphophallus paeonifolius* Tuber, mannose specific Agglutinin (AMTL) showed conserved mannose binding domains, agglutinating ability of rabbit erythrocytes and insecticidal efficacies. The 25 kDa dimeric protein was found to inhibit the survivability of hemipteran insects. It was shown that insecticidal AMTL binds to insect gut brush border membrane vesicle (BBMV) protein, conferring toxicity against target insects. It can be a promising candidate in preventing crop loss caused due to hemipteran insect attack[38].

2.10 CONSTRAINTS TO AROIDS RESEARCH, PRODUCTION AND UTILIZATION

The perishability and postharvest losses of root and tuber crops are the major constraints in the utilization of these crops. Edible aroids have a short shelf life period and this creates problems with the supply of new planting materials. Several simple, low-cost traditional storage methods are being followed by farmers in diverse parts of the world to store root and tuber crops in the fresh state. But these traditional storage systems are mostly suited for short-term storage and have partial success with long-term storage. In particular, storage at ambient temperatures is considered impossible due to very high incidence of fungal decay. Corms decay and become unfit for human consumption after a short period.

Proper storage is necessary for marketing these tubers beyond the harvest period. Root and tuber crops such as cassava and taro, suffers post-harvest losses estimated to be 30% caused partly by external agents, such as insects, rodents and moulds. Food losses occurring at these post harvest phases are most important in developing countries, due to poor infrastructure,
low levels of technology and low investment in the food production systems, resulting in a significant gap between gross production and the net availability to the consumer.

Betterment in post harvest technologies for preservation and processing of agri products would help in improving the returns to the farmers, which will aid in regulating the market infrastructure. These problems demand innovations in harvesting and storage technology that are appropriate to the smallholder farmers who cultivate these crops. Improvements in harvesting systems that reduce the occurrence of physical injury to corms while reducing the drudgery of hand harvesting will also be beneficial.

Post-harvest deterioration of *Amorphophallus* corms leading to visible signs of physiological deterioration are considered to have poor eating and processing qualities and leads to economic losses like corms remain unsold or sell at much discounted price. Therefore main reason for processing these corms into a variety of products is necessary to avoid these quality losses. Improvement in the processing technologies like converting corms into flour and starch is economical process and a way to preserve these corms throughout the year. Phyto-constituents characterization and development of products from these corms will definitely add value to this crop.

**2.11 VALUE ADDITION**

It is not only used as vegetables but recently several value added products like pickles, dried cubes, chips, thickening agents etc are also made and they are gaining popularity. Preparation of osmodehydrated slices from fresh corm [39]and bread from flour of *Amorphophallus paeoniifolius* corm flour (20 %)could substitute wheat flour in bread preparation[8]. Our studies confirmed the presence of phytoconstituents like alkaloids, tannins, phenols, carbohydrates, fat from peel where as cellulase and polyphenoloxidase from both corm and peel[40-41].

**2.12 PROCESSING OF RAW INGREDIENTS**

**2.12.1 Drying**

Drying is mainly removal of moisture from food to a certain level at which micro organisms are not able to grow is called drying, it can be done by these methods:
2.12.1.1 Sun Drying

In Sun drying method, food is directly exposed to sunlight. It is usually done in places where ample sunshine is available for long period. The dried product in this method is somehow inferior in quality. Excess production and specially grown crops may be preserved by natural drying methods for use until the next crop is grown and harvested. Shade drying is done for products which can lose their colour or may turn brown in direct sunlight. Like, Herbs, Green and red sweet peppers, chillies, green beans and okra.

2.12.1.2 Mechanical drying

This method involves of application of heat by a mechanical dryer under the controlled conditions of temperature, humidity and air flow.

2.12.1.3 Vacuum drying

In this method temperature of the food and the water removal rate are controlled by regulating the degree of vacuum and intensity of heat input.

2.12.1.4 Freeze drying

This method of drying involves sublimation process, i.e., rapidly freezing the food without passing through the liquid form of water by means of high vacuum and heat in the drying chamber causing minimal harm to the product. In this method, product is first frozen then water removed by vacuum and application of heat which occurs concurrently in same chamber.

2.12.1.5 Osmo-dehydration

Osmotic dehydration involves the removal of water by soaking fresh material in a heavy liquid sugar solution or strong salt solution and then the material is solar dried. The osmotic dehydration of fruits, meats and vegetables has been the aim of steady research attention during latest years as a valuable method to improve the economics of dehydration processes [42], [43] The use of elevated concentration of sugar binds the moisture and make the certain level of moisture in food which inhibits the micro organisms growth.

The concentration of salt causes high osmotic pressure and inhibits the growth of micro organisms as well as dehydrates the food as well as microbial cells.
Microwave drying, infra-red radiation drying, electric or magnetic field drying, superheated steam drying, explosion puffing, acoustic drying are some other novel drying technologies [44].

2.13 PHYTOCONSTITUENT CHARACTERIZATION

Plants contain thousands of constituents and are valuable sources of new and biologically active molecules. Phytochemicals are active constituent’s that are produced by plants.

2.13.1 Health-promoting phytochemicals

Bioactive compounds in plants are mainly secondary plant metabolites eliciting pharmacological or toxicological effects on biological systems. Studies have shown that natural products which are derived from food and medicinal plants acts as the potential sources of antioxidants.

**Flavonoids** are found in plenty of grains, vegetables, and fruits. The flavonoids present in soybeans, chickpeas and licorice may act a slightly like estrogen which might work against risk of breast cancer that depends on estrogen for its growth.

Researchers are studying flavonoids to see if they can reduce the risk of certain types of cancers and heart disease.

**Antioxidants** found in many plants like broccoli, brussels sprouts, cabbage, cauliflower, tomatoes, corn, carrots, mangos, sweet potatoes, soybeans and grains etc. protect our body's cells from free radicals which are unstable molecules created during normal cell functions. Pollution, radiation, cigarette smoke, and herbicides are common cause of free radicals in body. Free radicals can damage a cell's genetic parts and may trigger the cell to grow out of control. These changes may contribute to the development of cancer and other diseases.

**Carotenoids**, which are responsible for their orange colour in carrots, yams, cantaloupe, squash, and apricots, may help to reduce the risk of cancer.

**Anthocyanins**, which give grapes, blueberries, cranberries, and raspberries their dark color, have been shown in the laboratory to have anti-inflammatory and anti-tumor properties.

**Sulfides**, found in garlic and onions, may strengthen the immune system.
These people who eat mainly plant-based diets appear to have markedly decreased rates of certain types of cancers and heart disease. Some of the associations between specific phytochemicals and cancer risk reduction are very convincing, but additional research is needed. So far there is no decisive evidence that any phytochemicals will help reduce the risk of getting cancer. Therefore it is always recommended that eating a balanced diet having variety of vegetables, fruits, legumes and whole grains is essential for good health.

2.13.2 Carbohydrates

Carbohydrates are the major component of fruit, vegetables, play a major role in biological systems and correspond to more than 90% of their dry matter. From energy point of view carbohydrates stand for the most valuable food components. Adult daily intake should contain about 500 g carbohydrates. Photosynthesis process of green plants leads to the production of carbohydrates.

Carbohydrates contain following categories of molecules:

**Monosaccharides**: This group is the simplest carbohydrate also known as simple sugars. In general, the basic molecular formula is \((\text{CH}_2\text{O})_n\) with \(n\) generally being between 3 and 7 in living organisms. Examples include glucose, fructose and galactose.

The major function of monosaccharides is as a resource of energy for organisms and this energy is harnessed by the cell to do work. They are broken down quickly by the body and additionally act as building block for complex carbohydrates.

**Disaccharides**: These consist of two monosaccharides which are joined together by a covalent bond. Common examples of disaccharides are sucrose or table sugar \((\text{glucose-fructose})\), maltose \((\text{glucose-glucose})\) and lactose \((\text{glucose-galactose})\). The main function of disaccharides is as a nutritional source of monosaccharides. Many of the sugars found in foodstuffs are disaccharides.

**Oligosaccharides**: These are complex carbohydrates that consist of three to ten sugars. They are rich in vitamins and minerals and fibres, because of presence of fibres these are slower to digest than a simple carbohydrate.
**Polysaccharides**: These are also complex carbohydrates and are rich in vitamins, minerals and fibres having larger numbers of sugars than oligosaccharides. The two major polysaccharides that are important for physiology are **starch** (made exclusively by plants) and **glycogen** (made by animals). These two molecules are very similar in that they are **polymers** of glucose joined by 1-4 *alpha*-glycosidic bonds. Glycogen tends to be a bit more branched than starch but not quite as long.

Polysaccharides can also be used as structural components. The cell walls of plants and other things such as wood and paper are also formed by long polymers of glucose i.e. **cellulose**. The glycosidic bonds in cellulose are in the *beta* configuration which differs from starch. Because of this difference cellulose is not used as a nutrient.

**Starch** is one of the most abundant carbohydrates in root, cereal crops and green plants. This polysaccharide is produced by all green plants as an energy store. It is the most important carbohydrate in the human diet. The cereal grains (wheat, rice, corn, oats, and barley) as well as tubers such as potatoes, cassava, yams are rich in starch. Plants store glucose as the polysaccharide starch. Starch is a primary source of stored energy and consists primarily of D-glucopyranose polymers linked by $\alpha$-1, 4 and $\alpha$-1, 6 glucosidic bonds called amylose and amylopectin, respectively.

### 2.13.3 Applications of starch

Starch is gaining utmost attention in recent years because of its usefulness in various pharmaceutical, food and non-food based industrial products in native and modified form. Modification in the native starches are required to overcome the shortcomings like freeze thaw stability, solubility, paste clarity etc. Native starches are used as binder and disintegrant in solid dosage form. In recent years modified starch i.e. Pregelatinized starch like starch 1500 a unique pharmaceutical excipient is preferred in pharmaceutical industry. Native starch is utilized in the food industry in a different forms, like enhance paste consistency, thickening, smoothness and clarity and also to impart cold storage stability. Modified starches are used to encapsulate or, preserve the flavor of the food products and used in dairy products.
2.13.4 Fats

Generally fruit and vegetables contain very low level (below 0.5%) of fat. Fat is used by the body in much the same way as it uses carbohydrates. Fats are converted into energy by being split into fatty acids and glycerol. Fats are significant in way that they may provide stored form of energy, but not a necessity in the diet as far as a fuel source goes.

2.13.5 Organic acids

Fruit contains natural acids like citric acid, malic acid and tartaric acid. Citric acid is found in orange and lemon, malic acid in apples and tartaric acid in grapes. These acids provide the tartness, slow down bacterial spoilage of fruit and also lower the pH of food. Organic acid influence the colour of foods also since many plant pigments are natural pH indicators.

2.13.6 Nitrogen-containing substances

These compounds are found in plants as different combinations like proteins, free amino acids, amides, amines, nitrates, etc. Among these substances proteins are the most important compound having a colloidal structure and with heating, their water solution above 50°C one-way reaction makes them insoluble. From a biological point of view vegetal proteins are less valuable than animal ones because their composition does not contain all essential amino-acids.

2.13.7 Vitamins

Vitamins are defined as organic materials which must be supplied to the human body in small amounts apart from the essential amino-acids or fatty acids.

Vitamins function as enzyme systems which help the metabolism of proteins, carbohydrates and fats. The vitamins are conveniently divided into two major groups, fat-soluble and water-soluble vitamin. Fat-soluble vitamins are A, D, E and K. Their absorption by the body depends upon the normal absorption of fat from the diet. Water-soluble vitamins include vitamin C and several members of the vitamin B complex.
2.13.8 Enzymes

Enzymes are biological catalysts that help most of the biological reaction by the conversion of substrates into products by lowering the activation energy of the reaction.

In storage and processing of fruit and vegetable enzyme play important roles. The classes of enzyme responsible for that are hydrolases (lipase, invertase, tannase, chlorophylase, amylase, cellulase) and oxidoreductases (peroxidase, tyrosinase, catalase, ascorbinase, polyphenoloxidase). Since enzymes enter into a vast number of biochemical reactions in fruits and vegetable, they may be responsible for changes in flavour, colour, texture and nutritional properties. Today's consumer considers fresh food to be preferable for various obvious reasons, like being healthier or having a high nutritional value. Therefore, the need arises to check, or delay food spoilage and deterioration, which can be caused by various factors, such as oxidation, micro-organism growth, on-enzymatic and enzymatic browning.

Every enzyme has an optimal temperature and optimum pH where their activity is at maximum. Heating further than this optimal temperature deactivates the enzyme.

2.13.8.1 Polyphenol oxidase

Detrimental browning of foods, catalyzed by enzymes, is referred to as enzymatic browning involving the enzyme, polyphenoloxidase(PPO). Generally, the browning of foods is undesirable and reduces not only the appearance but the nutritional value of that particular food but in some cases, such as coffee and tea, brown appearance is desired quality characteristic. The colour of prunes and raisins is also attributed to PPO. However, in vegetables and fruits, browning reduce consumer appeal, thus decreasing the market value of the food. PPO plays a critical role in human functions also, like in eye, hair and skin pigmentation and PPO is specifically critical in the formation of the exoskeletons of insects. In general, browning results from both enzymatic and non-enzymatic oxidation of phenolic compounds. Browning usually impairs the sensory properties of products because of the coupled changes in colour, flavour and textures softening[45].

This unfavourable enzymatic browning occurring in many plants and vegetables are of great concern to Food Technologists and processors. Even if the discolouration of browning is not
a chemical quality defect[46], but due to less appealing appearance to consumers it reduces the market value of the fruits and vegetables. Active site of PPO comprises copper ions coordinated by three Histidine residues each.

On the basis of substrate specificity, number of active site copper ions and inhibitor action, PPOs are classified as

1. Tyrosinases
2. Catechol Oxidases
3. Laccases

| Table 2.1: Difference between Tyrosinase, Catechol oxidase and Laccase |
|---|---|---|
| **Enzyme commission Number** | **Tyrosinase** | **Catechol oxidase** | **Laccase** |
| E.C. 1.14.18.1 | E.C. 1.10.3.1 | E.C. 1.10.3.2 |
| Activity Type | Cresolase + Catecholase | Catecholase (usually o-diphenols as substrates) | Catecholase (more affinity for p-diphenols) |
| Cu ions in active site | 2 | 2 | 4 |
| Source and location | **Animals** - transmembrane (in melanosomes) | **Animals** - membrane bound | **Bacteria** - cytoplasm |
| | **Plants** - Thylakoids | **Plants** - chloroplast, soluble, membrane bound | **Fungi** - Extracellular and intracellular |
| | **Fungi** - Extracellular | **Plants** - chloroplast, soluble | **Plants** - chloroplast, soluble |
| | **Bacteria** - Extracellular | |
| Inhibitors | Tropolone, cinnamic acid and salicylhydroxamic acid. | Tropolone, cinnamic acid and salicylhydroxamic acid. | Azide, cyanide, thiocyanide, flouride and sulphydryl reagents. |
2.13.8.2 Cellulase enzyme

Cellulose is the most abundant organic biopolymer on earth with an estimated annual production of 180 billion tons in nature[48], [49]. Cellulase is a group of enzymes which hydrolyze the polysaccharide cellulose into soluble sugars. They are distributed throughout the biosphere such as plants, animals, and microorganisms. The cellulase enzymes have attracted considerable attention in recent years due to their great biotechnological and industrial potential. Cellulolytic activity is a multicomponent enzyme system and consists of three major components; exoglucanase, endoglucanase, and β-glucosidases. Each of these enzymes interacts with cellulose fibrils in a specific manner.

They have the following specificity:

1. Exoglucanase (1, 4-β-D-glucan) has multiple function it acts on the ends of cellulose chains to produce cellobiose as the main product.
2. Endoglucanase acts randomly on cellulose to produce oligosaccharides of variable sizes\[50\], [51].

3. \(\beta\)-glucosidases may be divided into three subgroups on the basis of substrate specificity:

(a) Cellobiases, which hydrolyze only oligosaccharides.
(b) Broad-specificity-\(\beta\)-glucosidases exhibit activity on many substrate types. They are the most commonly observed \(\beta\) glucosidases.
(c) Aryl-\(\beta\)-glucosidases, which have a strong affinity for aryl-\(\beta\) glucosides.

![Cellulose hydrolysis by a cellulase-system][52]

**Figure 2.3:** Cellulose hydrolysis by a cellulase-system\[52\]

2.13.9 Application of Poly phenol oxidase enzyme

2.13.9.1 Food industry
The natural colour and taste of the fruit juices is due to presence of phenol compounds and their oxidative products. Polymerization and oxidation of phenol derivatives and polyphenols,
leads to the colour and aroma change. PPO is used in beverage processing for the elimination of phenolics which are accountable for browning, haze formation and turbidity development in beer, wine and fruit juice[53]. Laccase is commonly used for stabilizing fruit juices and also helpful in removing excess oxygen in beer thus increasing shelf life of beer. Laccase treatment removes phenol as well as substrate-enzyme complex with the help of processes such as membrane filtration[53].

PPOs, particularly laccases are currently of interest in baking since they are able to cross-link biopolymers. Addition of laccase in the dough increases strength of gluten structures in dough and baked products are improved in crumb structure, product volume increases, and softness. PPO can be also used for the biosynthesis of antioxidants and food colorants[54].

2.13.9.2 Paper and pulp industry

Chlorine and oxygen-based chemical oxidants (e.g. ClO₂ and O₂) are generally used for the separation and degradation of lignin from woody tissues and pulp bleaching which is required for the preparation of paper at industrial level. Although these methods are very effective but have serious drawbacks such as disposal of chlorinated byproducts and loss of cellulose fibre strengths. These methods also have major environmental concerns associated with them because they lead to the release of toxic contaminants. The applications of laccases for the purposes of delignification and biobleaching have been found to be successful since they provide cleaner and milder strategies. For example, *Trametes versicolor* laccase has been reported to delignify Kraft pulp effectively in pilot plant scale[53].

2.13.9.3 Medicine and personal care

PPO has been reported to oxidize, polymerize and detoxify urushiol, a catechol- derivative toxin, responsible for causing poison ivy dermatitis, consequently reducing its effect. It has also been reported to oxidize iodide to iodine, a reagent widely used as a disinfectant[55]. According to a recent research, PPOs were found to inhibit the adhesion of *Streptococcus sobrinus* bacteria responsible from oral cavity formation on tooth surface [56]. Moreover, polyphenol oxidases can be used for the treatment of Parkinson’s disease by providing, conversion of L-tyrosine into L-DOPA that is used to supplement the insufficient amount of dopamine in Parkinson’s disease [57].

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2.13.9.4 Environment technology

The presence of hazardous phenolic compounds and their derivatives in industrial wastewaters from coal conversion, petroleum refining, wood preservation, paper, food, textile and chemical industries constitutes a big problem. Recent interest has focused on the use of peroxidases and polyphenol oxidases as an enzymatic approach for the removal of phenolics from industrial effluents[58]. In this aspect PPOs appear to be more advantageous because they require only molecular oxygen as oxidant to work. Recent developments are focusing on using enzymes for treatment of specific pollutants. Oxidoreductive enzymes such as peroxidases, laccases and polyphenol oxidases have the potential of degrading a broad range of aromatic pollutants and other substrates present in very low concentration[59].

PPOs prove to be better alternatives than peroxidases as they utilize free molecular oxygen as an oxidant. PPOs from potato (Solanum tuberosum), brinjal (Solanum melongena)[59], banana peel[60], have been efficiently extracted and used to decolorize various textile and non-textile dyes.

2.13.10 Applications of cellulase Enzyme

2.13.10.1 Food Applications

Cellulase enzymes have multiple applications in food industry like extraction and clarification of juices, bread production, and pigment extraction. Conversions of agricultural and food industries wastes to valuable sugars are the vast uses of cellulase enzymes [61]. Baking industry utilizes cellulase enzymes in common practice as natural additives[62]. The hydrolytic enzymes like xylanases and cellulases (β-glucanases) have gained popularity for being used in the baking industry to improve the dough-handling properties [63,-64 65]. CMCase and hemicellulase used in bread preparation and observed an increase of bread specific volume (18-19% ) [66].
The fruit juices containing vitamins and minerals play an important role in human health. Difficulties encountered in various steps in juice processing industry, especially in juice filtration, diverted attention to work out new methods to resolve this. Research was aimed on industrially suitable enzymes. The clarity and homogeneity of juices can be achieved by the complete removal of all suspended solids, i.e., polysaccharides (pectin, cellulose, hemicellulose, lignin, and starch), proteins, tannin, metals, and microorganisms as turbidity is undesirable from marketing perspectives [67]. Usually cellulases and pectinases are used in order to degrade pectins and polysaccharides in crude juice [68][69].

2.13.10.2 Pulp and Paper Industry

The mechanical pulping processes like refining and grinding of the woody raw material lead to pulps with more content of fines, bulk, and stiffness on the other hand the biomechanical pulping using cellulases enzyme results in substantial energy savings (20–40%) during refining and improves the hand-sheet strength properties [70],[71]. Hemicellulase enzymes like xylanase can enhance the bleaching property allowing reduction in the consumption of chlorine. Cellulases alone, or used in combination with xylanases, are beneficial for deinking of different types of paper wastes and release of ink from the fiber surface carried out by partial hydrolysis of carbohydrate molecules which helps in reduced alkali usage [72].

2.13.10.3 Textile applications

Traditional stonewashing of jeans involves treatment of material with pumice stone (1-2 kg/pair of jeans) and amylase-mediated removal of starch coating in large washing machines. Cellulases have been successfully used for the biostoning of jeans and biopolishing of cotton and other cellulosic fabrics. The advantages in the substitution of pumice stones by a enzyme based treatment contain less damage of fibres, increased productivity of the machines and less work-intensive and environment benign [73].
2.14 JUSTIFICATION OF RESEARCH PROJECT

Literature demonstrated that *A. paeoniifolius* is a very important crop in terms of export potential, great economic returns, ease and flexibility in management and multiplicity in uses with various medicinal properties. The misunderstanding of *Amorphophallus* calcium oxalate and negative attitude towards its products led to the negligence in research and development.

Although a lot of work is being done on *Amorphophallus paeoniifolius*, however, there has been very little interaction among researchers, farmers and users, which hampers expansion in promotion of the crop. There exists a big gap in researchers’ knowledge of phytoconstituent characterization, utilization of non edible part (peel) and food products development from *A.paeoniifolius*.

This study was intended at looking into these aspects and tried to come up with some solutions or recommendations which could lead to efficient utilisation of *Amorphophallus* products in industries.