Nutraceutical Profiling of Aril of

Blighia sapida K.D. Koenig

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CONTENTS

1. ABSTRACT

The Nutraceutical profiling of the aril of *Blighia sapida* was conducted by standard techniques. The preliminary phytochemical analysis revealed that the acetone extract and the distilled water extract of *B. sapida* aril showed more different classes of phytochemicals. The proximate composition, total phenolic and flavonoids, phytochemical constituents and antioxidant activity of *B. sapida* aril was estimated. The moisture content was found to be 72% in ripe aril and 42% in unripe aril. The crude fibre content of ripe aril and unripe aril was 3.7% and 2.2% respectively. The starch content in unripe aril and ripe aril was 12.3% and 21.7% respectively. The common biomolecules such as carbohydrate, protein and fat were present in adequate amounts. The unripe aril contains 2.34mg/g carbohydrate, 1.8mg/g protein and 6.7mg/g fat while the ripe aril contains 6.62mg/g carbohydrate, 4.3mg/g protein and 9.81mg/g fat. The nutritive value estimated for unripe aril and ripe aril was 120.6 KCal/100g and 148.4 KCal/100g respectively. The phenolic and flavonoid content of ripe aril was found to be 5.22mg/g and 2.8% whereas in unripe aril it was 4.25mg/g and 1.5%. The in vitro antioxidant assay by DPPH method and reducing power assay showed that the aril have remarkable antioxidant property. In vitro antidiabetic alpha amylase inhibition assay shows that unripe aril has an anti-hyperglycaemic effect. The ripe aril flour of *B. sapida* was extracted with petroleum ether using soxhlet apparatus for oil extraction. Average oil yield obtained in this study is 60% which indicates an elevated level of lipid content in ripe aril. The saponification number, acid value and iodine number of the oil was estimated and is 715.2mg KOH/g, 30.86% and 14.5 mg I_2 /g. The value estimated is almost similar to that of other edible oils compared like coconut oil, sesame oil, rice bran oil and sunflower oil. The peroxide value was 1100mgeq/g which is higher in amount when compared with the edible oils mentioned above. The total polar content of the oil was 20% and the lipase activity of the oil is similar to other edible oils. Hence it is safe for consumption. The study showed that the *B. sapida* oil and ripe aril could be used as a potential source for the functional ingredients.

2. INTRODUCTION

Plants can be found in all known parts of the earth. The importance of plants in the food chain dates back to ancient times. The terrestrial and aquatic ecosystem contains a lot of plants. They include algae, mosses, ferns, vines, herbs, shrubs, trees etc. So, plants are one of the major groups of living organisms that are essential to the function of the biosphere. The first human gathered wild plants for food. As settlements developed, food crops were cultivated leading to the selection of high-yielding cultivated varieties to feed the growing populations.

The essential foods produced by plants are carbohydrates, fats, and proteins each being of value in its own way to human and animal's metabolism. Growing archaeological evidence suggests that the diets of Palaeolithic people were not as meat-oriented as often portrayed. Plant foods probably represented 50% or more of their diets.

Plants are irreplaceable food resources for humans. Although many synthetic chemicals can replace other plant derived materials, there is no substitute for plant derived food. People depend upon plants to satisfy their basic human needs such as food, clothing, shelter, and medicine. To date, these basic human needs are growing rapidly because of a growing world population.

In many cultures, the distinction between food and medicine is obscure. Food provides calories and nutrients, but they also contain many chemical compounds that can prevent or alleviate disease. Food has another significant value-food and meals have important ritualistic and symbolic values. Along with language and religion, food ways are one of the defining elements of a culture.

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi, and herbivorous mammals. Many of these phytochemicals have effects on long term health when consumed by humans and can be used to effectively treat human diseases. In India, thousands of plant species are known to have medicinal values. Medicinal plants are valuable natural resources and regarded as potentially safe drugs.

Advantages of plants-based food over animal origin

People become vegetarians for many reasons, including health, religious convictions, concerns about animal welfare or the use of antibiotics and hormones in livestock, or a desire to eat in a way that avoids excessive use of environmental resources. Traditionally, research into vegetarianism focused mainly on potential nutritional deficiencies, but in recent years the pendulum has swung the other way, and studies are confirming the health benefits of meat-free eating. Nowadays, plant-based eating is recognized as not only nutritionally sufficient but also a way to reduce the risk for many chronic illnesses. According to the American Dietetic Association (2009), "approximately planned vegetarian diets, including total vegetarian or vegan diets, are healthful, nutritionally adequate and may provide health benefits in the prevention and treatment of certain diseases".

Recent studies have linked non- vegetarian food consumption to increased risk of cancers. Consumption of high amounts of non-vegetarian foods, particularly red meat can also work as one of the primary reasons for an increased blood pressure, diabetes, kidney disorders, weight gain and more risk of heart ailments (Rothgerber, 2014). Red meat and processed meats are associated with increased mortality, according to the National Institute of Health (2012). Just because many vegetarians have lower risks does not mean that a vegetarian eating plan is automatically healthier than a diet that includes meat. The key to forming any healthy eating plan, vegetarian or not, is including a variety of nutritious items from every major food group that will satisfy all of your nutritional needs.

Importance of phytochemical properties and analysis of Nutritional quality

Plants are potent biochemists and have been used as components of phytomedicine since time immemorial. Plant based natural constituents can be derived from any part of a plant like bark, leaves, flowers, roots, fruits, seed, etc., i.e., any parts of the plant may contain active components (Abayomi *et al*, 2013). The beneficial medicinal effects of plant materials typically result from the combination of products such as alkaloids, phenols, proteins and amino acids, carboxylic acid, coumarins, flavonoids, quinines, resins, saponins, sterols, tannins, xanthoproteins, sugars, etc. presents in the plant. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. These compounds possess biological properties such as antiapotosis, anti-ageing, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improving of the endothelial functions as well as inhibition of angiogenesis and cell proliferation activity.

Malnutrition is a very serious global health problem among the rural populace, especially the pregnant women and children in developing countries. Food contains essential ingredients for sustenance of plants and animals. Small but mighty, seeds are packed with life-enhancing nutrients such as protein, carbohydrate, fiber, vitamins and fatty acids that can help the body fight diseases and promote good healthy living. The estimation of nutrient intake from food consumption requires reliable data on food composition. These data are also the fundamentals of food-based dietary guidelines for healthy nutrition, containing the necessary information on food sources for different nutrients. Furthermore, food composition tables can provide information on chemical forms of nutrients and the presence and amounts of interacting components, and thus provide information on their bioavailability.

Antioxidants are the substance that delays or inhibits oxidative damage to a target molecule. The characteristic feature of an antioxidant is ability to scavenge the free radicals due to their redox hydrogen donors and singlet oxygen quenchers. The free radicals can be scavenged by the natural and synthetic antioxidants. But the usages of these synthetic antioxidants are now replaced because the natural antioxidants could be considered as safer without any side effects. Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiology studies have indicated

the frequent consumption of natural antioxidants is associated with a lower risk of cardio-vascular disease and cancer.

About the plant source- *Blighia sapida* K. D. Koenig

Fig 1.1: Habitat of *B. sapida* tree

The *B. sapida*, also known as ankye, achee, akee, acke apple or aye. It is a fruit of the Sapindaceae (soapberry) family, as are the lychee and the longan. It is native to tropical West Africa. The scientific name honours Captain William Bligh who took the fruit from Jamaica to the Royal Botanic Gardens in Kew, England in 1793 (Lancashire, 2004). The English common name is derived from the West African Akan akye fufo.

Although having a long-held reputation as being poisonous with potential fatalities, the fruit arils are renowned as delicious when ripe, prepared properly, and cooked, and are a feature of various Caribbean cuisines. *B. sapida* is the national fruit of Jamaica and is considered a delicacy.

BOTANICAL FEATURES

Scientific classification based on APG IV

Kingdom	:	Plantae
Clade	:	Tracheophytes
Clade	:	Angiosperms
Clade	:	Eudicots
Clade	:	Rosids
Order	:	Sapindales
Family	:	Sapindaceae
Genus	:	Blighia
Species	:	*Blighia sapida* K.D. Koenig

B. sapida is an evergreen tree that grows about 10 meters tall, with a short trunk and a dense crown. The leaves are pinnately compound of 15–30 centimetres long, with 6–10 elliptical to oblong leathery leaflets. Each leaflet is 8–12 centimetres long and 5–8 centimetres wide. The inflorescences are fragrant, up to 20 cm long, with unisexual flowers that bloom during warm months. Each flower has five greenish-white petals, which are fragrant.

The fruit is pear-shaped and has 3 lobes (2 - 4 lobes are common). When it ripens, it turns from green to a bright red to yellow-orange, and splits open to reveal three large, shiny black seeds, each partly surrounded by soft, creamy or spongy, white to yellow flesh — the aril having a nut-like flavour and texture of scrambled eggs. The fruit typically weighs 100–200 grams. The tree can produce fruit throughout the year, although January–March and October–November are typically periods of fruit production.

Fig 1.2: Leaves, upper and lower surface

Fig 1.3: Inflorescence

Fig 1.4: Fruit as it splits

Fig 1.5: Ripe fruit Opened

Fig 1.6: Ripe seeds with their arils (dorsal view and in longitudinal section)

History and Culinary Use

B. sapida is the national fruit of Jamaica while *B. sapida* and salt fish is the official national dish of Jamaica. The *B. sapida* is allowed to open fully before picking in order to eliminate toxicity. In Caribbean cooking, they may be cooked with codfish and vegetables or they may be added to stew, curry or soup with seasonings.

Cultivars

There are up to as many as forty-eight cultivars of *B. sapida*, which are grouped into either "butter" or "cheese" types. The cheese type is pale yellow in colour and is more robust and finds use in the canning industry. The butter type is deeper yellow in colour, and is more delicate and better suited for certain cuisine.

Toxicity

The unripen aril and the inedible portions of the fruit contain the toxin hypoglycin A and hypoglycin B, known as "soapberry toxins". Hypoglycin A is found in both the seeds and the arils, while hypoglycin B is found only in the seeds. Minimal quantities of the toxin are found in the ripe arils. In the unripe fruit, depending on the season and exposure to the sun, the concentrations may be up to 10 - 100 times greater.

These two molecules are converted in the body to methylene cyclopropylacetic acid (MCPA), and are toxic with potential lethality. MCPA and hypoglycin A inhibit several enzymes involved in the breakdown of acyl CoA compounds, often binding irreversibly to coenzyme A, carnitine and carnitine acyltransferases I and II, reducing their bioavailability and consequently inhibiting beta oxidation of fatty acids. The stored glucose is consequently depleted leading to hypoglycaemia, and to a condition called Jamaican vomiting sickness. These effects occur only when the unripe aril (or an inedible part of the fruit) is consumed (Hassall *et al*, 1954).

Though *B. sapida* is used widely in traditional dishes, research on its potential hypoglycin toxicity has been sparse and preliminary, requiring evaluation in well-designed clinical research to better understand its pharmacology, food uses, and methods for detoxification.

Economic Importance of *B. sapida*

- *Blighia sapida* is widely used for the treatment of yellow fever, epilepsy and oedema, and as a laxative and diuretic.
- It is widely used as an antibacterial agent.
- Sap from terminal buds is instilled in the eyes to treat ophthalmic and conjunctivitis.
- Of the oils investigated, *B. sapida* aril oil had the highest carotenoid content. Its free radical scavenging property was intermediate to that of coconut oil and soybean oil. Several characteristic peaks were observed in the NMR and FTIR spectral data which confirms that oleic acid is the major fatty acid present in *B. sapida* aril oil. So the aril oil is considered for commercial food applications.
- The fruit has various uses in West Africa and in rural areas of the Caribbean Islands, including use of its "soap" properties as a laundering agent or fish poison.
- The fragrant flowers may be used as decoration or cologne, and the durable heartwood used for construction, pilings, oars, paddles and casks.
- In African traditional medicine, the ripe arils, leaves or bark were used to treat minor ailments.
- *B. sapida* aril is used as an antiulcer agent and it can also be used for curing type II diabetes.

Chemical property of Oils

Fats and oils are an essential part of the human diet. Since at least the 1930s, it has been realized that not all fats are created equal in terms of human nutrition. Studies in rats determined that certain polyunsaturated fatty acids (PUFA) derived from plants, namely, linoleic acids which were necessary to sustain life in animals. The quantity of any oil is indicated by some physical and chemical properties. The specific values of some of these properties provide an indication of both the nutritive and physical quality of the oil. These properties include iodine value, peroxide value, saponification value, saponifiable value, free fatty acid etc., The exposure of oils to either a source of heat, light or moisture can alter some of the quality indicators. The extent of alteration depends on the duration of exposure, temperature and condition of storage. Excessive heating of the oil results in polymerization. As oils and fats undergo heating in the frying process, various decomposition products are formed. Some of these products are volatile and others are non- volatile. The non-volatile decomposition products include polar compounds. The polar materials include all partially oxidized triglycerides, lipids and other

materials which are soluble/ emulsified/ suspended particulates in the frying oil (Al-Kahtani, 1991). The toxicity of these compounds is associated with several diseases such as hypertension, atherosclerosis, Alzheimer's diseases, liver disease etc.,

The determination of polar compounds is one of the most reliable methods for monitoring the quality changes in oil during the frying process and reflects the degradation of the oil after repeated use.

Significance of the Work

Malnutrition is a very serious global health problem, especially in the case of children and pregnant women of rural populations. Disease resistance of humans can be achieved by the intake of nutrient rich food. The high amount of carbohydrate, protein, lipid, starch of the plant parts provides energy and also these biomolecules can control the serious effect of malnutrition.

Presence of antioxidant molecules and secondary metabolites of each plant in the earth has the ability to control the diseases that attack the host plants and the consumers. Medicinal plants have been used in health care from ancient times. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines.

The study on analysis of the *B. sapida* aril and the physicochemical properties of the oil gives a lot of information about their medicinal and nutritional properties.

3. AIMS AND OBJECTIVES

Aims

The present works aims to analyse the proximate composition, nutritive content, invitro antioxidant and antidiabetic potential of *B. sapida* aril and also the physicochemical properties of the aril oil.

Objectives

The present work aims on the following objectives.

- Collection of *B. sapida* ripened aril and extract the bioactive principles using different solvents like Ethyl acetate, Chloroform, Distilled water, Ethanol and Acetone.
- Preliminary phytochemical analysis for the detection of various components present in the *B. sapida* aril.
- To analyse the proximate composition between ripe and unripe aril.
- Comparative analysis of the nutritional content of ripe and unripe aril.
- To analyse the total phenol content (TPC) and flavonoid content in ripe and unripe aril.
- To analyse the *invitro* antioxidant activity of ripe and unripe aril.
- To analyse the anti-diabetic property of ripe and unripe aril.
- Extraction and estimation of oil from the ripened aril.
- To analyse the chemical properties of extracted oil and estimation of Total Polar Materials using Column chromatography and its comparison with other edible oils.

4. REVIEW OF LITERATURE

The *B. sapida* is indigenous to the forests of the Ivory Coast and Gold Coast of West tropical Africa where it is little eaten but various parts have domestic uses. In Ghana, the fruiting tree is admired as an ornamental and is planted in villages and along streets for shade. The *B. sapida* was brought to Jamaica in 1793 by the renowned Captain Bligh to furnish food for the slaves. It was readily adopted and became commonly grown in dooryards and along roadsides and, to some extent, naturalized. The arils still constitute a favorite food of the island and the fruit is featured in a calypso despite the health hazards associated with it. Canned arils are exported to the United Kingdom where they are welcomed by Jamaican immigrants. Importation has been banned by the United States Food and Drug Administration.

The toxicity of the *B. sapida* was long misunderstood and believed to reside in the membranes attaching the arils to the jacket, or only in the overripe and decomposing arils. There have been intensive clinical and chemical studies of the *B. sapida* and its effects since 1940, and it is now known that the unripe arils contain hypoglycin, a-Amino-B-(2-methylene cyclopropyl) propionic acid, formerly called hypoglycin A. This toxic property is largely dispelled by light as the jacket opens. When fully ripe, the arils still possess 1/12 of the amount in the unripe. They contain hypoglycin and its y-glutamyl derivative, y-L-glutamyl a-Amino-B-(2-methylene cyclopropyl) propionic acid, formerly called hypoglycin B. The latter is half as toxic as the former (Hassall *et al*, 1954).

In Brazil, repeated small doses of an aqueous extract of the seed has been administered to expel parasites. The treatment is followed by a saline or oily purgative. Cubans blend the ripe arils with sugar and cinnamon and give the mixture as a febrifuge and as a treatment for dysentery. On the Ivory Coast, the bark is mixed with pungent spices in an ointment applied to relieve pain. The crushed new foliage is applied on the forehead to relieve severe headache. The leaves, crushed with salt, are poultice on ulcers. The leaf juice is employed as eye drops in ophthalmic and conjunctivitis. In Colombia, the leaves and bark are considered stomachic. Various preparations are made for treatment of epilepsy and yellow fever (Odutuga *et al*, 1974).

Ingestion of soapberry fruit toxins hypoglycin A and methylene cyclopropyl glycine has been linked to public health challenges worldwide. In 1976, over 100 years after Jamaican Vomiting Sickness (JVS) was first reported, the cause of JVS was linked to the ingestion of the toxin hypoglycin A produced by *B. sapida* fruit. A structural analogy of hypoglycin A, methylene

cyclopropyl glycine (MCPG), was implicated as the cause of Acute Encephalitis Syndrome (AES) (Samantha et al, 2015). Much of the evidence linking hypoglycin A and MCPG to these diseases has been largely circumstantial due to the lack of an analytical method for specific metabolites.

An analytical approach was conducted to identify and quantify specific urine metabolites for exposure to hypoglycin A and MCPG. The metabolites are excreted in urine as glycine adducts methylenecyclopropyl cyclopropylacetyl-glycine (MCPA-Gly) and methylene cyclopropylformyl-glycine (MCPF-Gly). These metabolites were processed by isotope-dilution, separated by reverse-phase liquid chromatography, and monitored by electrospray-ionization tandem mass spectrometry. The analytical response ratio was linearly proportional to the concentration of MCPF-Gly and MCPA-Gly in urine from 0.10 to 20 μg/mL with a correlation coefficient of $r > 0.99$. The assay demonstrated accuracy ≥ 80 % and precision ≤ 20 % RSD across the calibration range. This method has been applied to assess exposure to hypoglycin A and MCPG as part of a larger public health initiative and was used to provide the first reported identification of MCPF-Gly and MCPA-Gly in human urine. (Johannes *et al*, 2017)

B. sapida extracts showed antimicrobial activity against the tested microorganisms at varying levels. Both plant parts could be the best form of treatment to reduce the prevalence of infections caused by microorganisms. In addition, more research can be carried out on this plant to know the most active constituents of the plant responsible for antimicrobial activity; these active constituents can be isolated to develop new drugs which can be used for treatment of infections caused by microbes. Therefore, being able to identify plants and their active constituents that are potent against microorganism will be a breakthrough to solving the problem of emergence of resistance by microorganisms to antibiotics as new drugs could be formulated.

The antimicrobial activity of the plant material was carried out using the Agar well diffusion method. The extracts were tested against three strains of gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus* and *Salmonella typhi*) and three strains of gram negative bacteria (*Streptococcus pneumoniae, Escherichia coli* and *Klebsiella pneumoniae*) isolated from the clinic and characterized. Results of antimicrobial activity of *B. sapida* extracts confirmed a broad spectrum of activity on all the bacteria tested by aqueous, chloroform, hexane and ethanol, hence, the plant should be explored for the formulation of drugs to treat

infectious diseases caused by microorganisms. The two extracts did not exert antifungal effect on any of the tested fungal species at all concentrations but exhibited activity against *S. aureus* and *B. subtilis* but not against *E. coli* and *S. dysenteriae*. It was observed that the stem bark extract was more potent than the leaf extract (Ubulom *et al*, 2013).

In West Africa, the fruit, seed, leaf and stem of *Blighia sapida* are commonly used as a remedy against a variety of diseases, including diabetes mellitus. Oluwafemi *et al*, (2016) investigated the ameliorative potential of B. stem bark ethanol extract against pancreatic β-cell dysfunction in diabetic rats. Diabetes was induced by intraperitoneal injection of alloxan (65 mg/kg body weight) for 21 days, and orally administered with glibenclamide (5 mg/kg body weight), 50-150 mg/kg body weight of *B. sapida* stem bark ethanol extract once daily for 21 days. The blood glucose levels of rats induced with alloxan were significantly and gradually reduced ($p<0.05$) in *B. sapida* stem bark ethanol extract treated animals at the dose of 50-150 mg/kg body weight, and in glibenclamide-treated animals. The significant increase in the lipid peroxidation (malondialdehyde), homeostasis model assessment-insulin resistance scores (HOMA-IR) and decrease in serum insulin, pancreatic β-cell scores as well as antioxidant marker enzymes in untreated diabetic rats compared to normal control rats were reversed by the *B. sapida* stem bark ethanol extract and glibenclamide. Similarly, histopathological changes in the pancreas were also reversed by the extract and glibenclamide. However, these effects were most prominent in the animals treated with 150 mg/kg body weight of *B. sapida* bark. These findings indicate that *B. sapida* stem bark possesses anti-hyperglycemic activity and exhibits ameliorative potential in managing diabetes. (Oluwafemi *et al*, 2016).

Several prospective medicinal plants for peptic ulcer treatment have been studied and reported in literature globally. Lakshmi *et a*, reported that due to their folkloric use in the treatment of peptic ulcers and availability in Ghana, *Blighia sapida* K.D. Koenig. has been used as a product for the management of peptic ulcer disease at the CPMR out-patient clinic in Ghana along with other plants.

Adedosu *et al,* (2018). also evaluated the aqueous leaf extract of *Blighia sapida* K.D. Koenig. on ethanol-induced gastric ulcer in male Wister rats were animals treated with the extract at doses of 400 mg/kg body weight showed that *Blighia sapida* K.D. Koenig. leaves showed ameliorative, antiulcer, and anti-inflammatory activities.

A study conducted by Oreagba *et al* (2013), to analyze the antiulcer activity of the hydro ethanolic leaf extract of *B. sapida* using absolute HCl-ethanol, ethanol, cold-restraint stress,

indomethacin, and pylorus ligation ulceration in rats showed that the extract produced dose-relative antiulcer effects. This was evident by the significant protection of the gastric membrane. The hydro ethanolic leaf extract also markedly reduced the ulcer index in the cold-restraint stress and pyloric ligation-induced ulcer models compared to cimetidine and omeprazole standard drugs. Also, the levels of the pH, gastric juice volume, free acidity, and acidity determined in the ulcer model induced by pylorus ligation demonstrated that pretreatment with the hydro ethanolic leaf extract of *B. sapida*, safeguarded gastric mucosa by marked reduction of acid secretion and the ulcer index in gastric membrane injury. *B. sapida* may therefore have anti secretory, gastro protective, and free radical scavenging properties.

Oil extracted from the ripe *B. sapida* aril was characterized by the classical titrimetric and gravimetric analyses by using arachis oil and/or oleic acid was used as reference. following the British Pharmacopoeia procedures. The extraction and purification method produced 37.0 ± 4.9%, on a dry weight basis, of bright-yellow oil with characteristic roasted *B. sapida* seed. Acid, ester, hydroxyl and saponification values were 1.83 (±0.01) mg KOH/g, 64.52 (±0.18) mg KOH/g, 28.01 (±0.04) mg KOH/g and 743 (±0.19) mg KOH/g respectively and its specific gravity was 0.905 (±0.008) while the optical rotation was 1.453. So the results indicates that the sample has comparable specific gravity, viscosities and true density values as arachis oil BP. On the other hand, it contains higher levels of saponifiable matters, free acid and hydrolysable matters than arachis oil. Thus the characteristic properties of *B. sapida* oil suggest potential for its application as a pharmaceutical base and may satisfy some of the deficiencies of arachis and, possibly, some other vegetable oils (Elizabeth *et al*, 2011).

The study conducted by Tsado *et al,* (2018) shows that the physicochemical properties and fatty acid profile of oil extracts of aril had the highest oil yield of 47.05±0.54 %. The iodine values of the oils ranged from 63.72±2.43 to 116.54±1.00 mgI2/g while the acid values ranged from 4.91±0.16 to 9.02±0.34 mg KOH/g. On the other hand, the peroxide values ranged 5.05±0.21 to 9.44±0.09 mEq/kg while the saponification values ranged from 175.23±2.52 to 193.73±1.85 mg KOH/g. The fatty acid profile revealed that the oils contain more unsaturated fatty acids. Oleic acid (45.18%), 9-octadecenoic acid (51.25%) and oleic acid (89.95%) were the most abundant fatty acid present in the aril, seed and pod oils respectively. So the results obtained from the study showed that oils from the various parts of the fruit, upon further processing can be used as edible oil and for various industrial applications.

The mature *B. sapida* fruit is a rich source of lipids (Goldson *et al*, 2014). There have been several reports on the fatty acid profile of the aril of the fruit. Subsequent studies from different researchers utilizing various analytical techniques have consistently detected oleic acid as the primary fatty acid present (Emanuel *et al*, 2013; Goldson *et al*, 2014; Grande-Tovar *et al*, 2019). Analytical techniques employed in analysis of the *B. sapida* aril oil which detected oleic acid as the primary fatty acid present were inclusive of gas chromatography, gas chromatography mass spectrometry, mass spectrometry, Fourier transform infrared spectroscopy and nuclear magnetic resonance spectroscopy (Emanuel *et al*, 2013; Goldson *et al*, 2014; Goldson-Barnaby *et al*, 2018). Other fatty acids detected in the aril of the fruit included palmitic acid and stearic acid (Goldson-Barnaby and Williams, 2017). Research conducted by Goldson *et al*, (2014) and Goldson-Barnaby *et al*, (2018) over different time periods and fruits from various locations across the island of Jamaica has consistently shown oleic acid as the major fatty acid present (Goldson *et al*, 2014; Goldson-Barnaby *et al*, 2018). Initial reports suggesting that linoleic acid was the primary fatty acid had sparked concerns that *B. sapida* may be a contributing factor to the high incidence of prostate cancer in Jamaica. Oleic acid, the major fatty acid present in the aril is however associated with a decreased risk of prostate cancer (Goldson *et al*, 2014).

Carotenoids are lipid soluble terpene derivatives which contribute to the yellow color and antioxidant properties of *B. sapida* (Goldson-Barnaby *et al*, 2018). Carotenoids are nutritionally significant as they are the plant form of vitamin A. A study conducted on the carotenoid content and free radical scavenging activity of *B. sapida* aril oil found that it contained small quantities of carotenoids and exhibited free radical scavenging activity. When the β-carotene content of *B. sapida* aril oil (21 ± 0.2 ppm) was compared to that of soybean oil (1.39 ± 0.1 ppm) and coconut oil (0.34 ± 0.1 ppm), two of the more popularly used oils within Jamaica, the *B. sapida* oil was found to be a better source of carotenoids as the others had only trace amounts (Goldson-Barnaby *et al*, 2018).

Glucose, fructose, sucrose and short chain fructo oligosaccharides were detected in the *B. sapida* aril (Lopez and Benkeblia, 2017). FTIR analysis confirmed that sucrose is the predominant carbohydrate (Goldson-Barnaby and Williams, 2017). The seeds are rich in starch (Goldson-Barnaby and Williams, 2017). On a dry weight basis, the seeds contained 43.3% starch (Abiodun *et al*, 2015). *B. sapida* aril contained a higher percentage of ash (14.3 %) compared to the seeds (2.5 %) (Goldson-Barnaby and Williams, 2017) and is a source of the minerals, phosphorus, calcium, magnesium, potassium, sodium, iron and zinc.

The study conducted on the health and nutritional benefits of edible arils of the *B. sapida* showed that the moisture, crude fat, crude protein, crude fibre, ash, carbohydrate and energy content were in the range of 48.3-52.20%, 51.60-56.66%, 10.94-11.67%, 3.63-3.88%, 8.01-8.56%, 14.41-20.62% and 590.67-614.26 kcal/100g, respectively. The *B. sapida* arils also contained appreciable minerals (Ca, P, Mg, Na, K and Zn) with K (425.10-475.71 mg/100g) being the highest while Zn (1.95-2.08 mg/100g) was the least mineral. The total phenolic content was 5235.04±103.9 and 5175.38±178.46 mg GAE/100g in oven-dried and freeze-dried samples, respectively. The vitamin C content was 29.6 and 35.7 mg/100 g whereas antioxidant activity was 66.0 and 29.4 % DPPH inhibition (with trolox equivalence of 91.0±9.4 and 40.6±0.6 µM TE/g) in the oven and freeze-dried *B. sapida* arils, respectively. Drying method had an effect on the phytochemicals and antioxidant activity of the *B. sapida* flour sample. So the findings suggest that *B. sapida* aril had considerable total phenols content and antioxidant activity, which implies that the fruit aril has the potential for application in food systems to maintain food quality (Veronica *et al,* 2014).

Rabiat *et al (*2013) evaluated the antioxidant and anti-inflammatory activities of ethanol extract and fractions of *B. sapida* stem-bark using *in vitro* methods, and says that its stem exhibits antioxidant and anti-inflammatory properties. Antioxidant studies revealed that the ethyl acetate fraction displayed superior activity with an IC50 = 0.09 ± 0.03 mg/mL DPPH, and values of 146.96 ± 3.81 ascorbic acid equivalent (AAE) mg/g and 359.20 ± 4.98 AAE mg/g for FRAP and TAC.

The Ethanol Extract and the fractions of *B. sapida* stem-bark exhibited lethality against brine shrimp. The extract and its fractions contained active constituents supporting its medicinal values. The study revealed 13 active constituents, of which one is prominent in EAF by GC-MS analysis. The identified compounds with various biological activities indicate the medicinal value and wide ethno-medicinal use of the plant. Hence, extract of *B. sapida* stem-bark could be suggested for use in the synthesis of drugs with potential new mechanism of action to combat the menace of drug resistance. (Adekola *et al,* 2020).

The Methanolic extract of fruits of *B. sapida, Vitex doniana* L. and *Vitellaria paradoxa* L. were screened by Rabiat *et al,* (2013) for their phytochemical constituents and in vitro antioxidant properties using standard procedures. This study reveals the presence of alkaloids, tannins, saponins, flavoniods and phenols in all the fruits studied. So, all the fruits that were studied showed antioxidant activity in a dose dependent manner. From the results *Blighia sapida*

extract showed the highest 1,1-diphenyl-2-picrylhydrazyl (DPHH) free radical scavenging activity (78 ± 0.01%) and reductive potential compared to other extracts. *Vitellaria paradoxia* L. showed the highest capacity to inhibit lipid peroxidation (72%) than other fruit extracts while *Vitex doniana* L. had the lowest effect in inhibition of lipid peroxidation (60.7 ± 0.05%) (Rabiat *et al*, 2013).

Anupama and Sunil kumar (2019) reported the presence and the quantitative value of protein, carbohydrate, ascorbic acid, flavonoids, phenolic compounds, alkaloids, and minerals like Ca, Mg, P.

Phytochemical properties and fatty acid composition of ripe and unripe *B. sapida* seed (mg/100g) contains tannins 4662.83±15.4, phenols 317.20±0.89 and flavonoids 5.17±0.09 which was more in the ripe *B. sapida* seeds while alkaloids 0.48±0.02 and saponins 4208.33±17.61 were higher in the unripe *B. sapida* seeds. The ripe and unripe *B. sapida* seeds had an average oil yield of 15.61±0.01% and 14.05±0.02% respectively. *B. sapida* seeds can be used in the production of therapeutic agents and industrial oil (Emmanuel *et al*, 2014).

Oluba *et al* (2021) studied on the effect of drying method on the composition as well as the antioxidant activity of oven-dried and sun-dried *B. sapida* aril flour and oil showed that the Sun-dried *B. sapida* aril flour had significantly higher protein and fat content compared to the oven-dried flour. The findings of this study concluded that sun-drying is recommended for both nutritional purposes and other health-promoting usage such as antioxidants, over oven-drying

Seymour *et al,* (2019) investigated on the factors affecting the production of somatic embryos in *Blighia sapida* by using M S Medium showed that the leaf explants grown on media supplemented with the different combinations of 2,4-D and BAP formed callus, but they were non-embryogenic, while explants were not responsive on TDZ-supplemented media. When globular protuberances (GPs) were cultured on media containing TDZ and abscisic acid (ABA), gave rise to the highest number of somatic embryos.

5. MATERIALS AND METHODS

5.1: Collection of *B. sapida* aril

The *B. sapida* aril was used for the analysis of proximate composition, nutritional content, antioxidant potential and oil extraction. The *B. sapida* aril were collected from the tree in my front yard [redacted]

5.2: Extract preparation of ripened *B. sapida* aril

a. Solvents used

Organic solvents such as Acetone, Ethyl acetate, Chloroform, Ethanol, and Distilled water were employed for the extraction of different bioactive compounds.

b. Extraction

In this study, the plant metabolites were extracted using cold extraction (Percolation). About 8g of the cleaned, fresh sample was transferred into clean screw cap bottles of 50ml capacity. 40ml of various solvents were added and soaked it for a week separately

5.3: Preliminary Phytochemical analysis

The extract using different solvents were screened for the qualitative analysis of different classes of natural compounds, using the methodology of *Sofowora* (1982) and Kepem (1986). The major pharmaceutically valuable compounds, investigated by the present studies were:

5.3.1: Detection of Alkaloids

Extracts were dissolved individually in dil.HCI and filtered and then subjected to the following tests.

a) Hager's Test- Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

b) Dragendoff's Test- Filtrates were treated with I ml of Dragendoff's reagent. Formation of reddish orange precipitation indicated the presence of alkaloids.

5.3.2: Detection of Carboxylic acids

1 ml of each of various extracts was separately treated with a few ml of saturated solutions of sodium bicarbonate. Observation of effervescence (due to liberation of CO_2) indicated the presence of carboxylic acids.

5.3.3: Detection of Coumarins

1 ml each of alcoholic extracts was treated with alcoholic 10% NAOH solution. Production of dark yellow colour indicated the presence of Coumarins.

5.3.4: Detection of flavonoids

a) Alkaline reagent Test - Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour which becomes colourless on addition of dilute acid indicates the presence of flavonoids.

b) Lead acetate Test - Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitates indicate the presence of flavonoids.

2.5 ml of each of the various extracts were separately dissolved in 1 ml each of alcohol (stock solution) and subjected to the following test.

c) Ferric chloride Test- I ml each of stock solution was added with a few drops of neutral $FeCl_3$, solution, Formation of blackish red indicated the presence of flavonoids.

5.3.5: Detection of Phenols

a) The extract (0.5 ml) was dissolved in 5 ml of distilled water. To this, a few drops of neutral ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

b) Ferric chloride Test - A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

5.3.6: Detection of protein and amino acids

5 ml each of the various extracts were dissolved in 5 ml of water separately and were subjected to the following tests.

a) Biuret test - 1 ml of each of the various extracts was warmed gently with 10% NaOH solution and a drop of diluted $CuSO_4$, solution. Formation of reddish violet colour indicated the presence of proteins and amino acids

b) Ninhydrin test- 1 ml each of the various extracts was separately treated with a few drops of ninhydrin solution, Change in colour showed the presence of proteins and amino acids.

5.3.7: Detection of Quinones

a) 1 ml of the various extracts was separately treated with alcoholic KOH solution. Quinones give colouration ranging from red to blue

b) A small amount of extracts was treated with con. HCl and observed for the formation of yellow precipitation (or colouration)

5.3.8: Detection of Resins

One ml of various extracts was diluted with water. Formation of bulk black precipitate indicates the presence of resins.

5.3.9: Detection of Saponins

a) Froth Test - Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of Saponins.

b) Foam Test - 0.5 gm of extracts was shaken with 2 ml of water. If foam produced persists for few minutes indicates the presence of Saponins.

5.3.10: Detection of Steroids and Phytosterols

5 ml of each of various extracts were dissolved in 5 ml of chloroform separately (stock solution) and was subjected to the following test.

a) Salkowski Test - 1 ml each of conc. H_2SO_4 was added to the stock solution and allowed to stand for 5minutes after shaking. Turning of golden yellow colour in the lower layer indicated the presence of steroids and Phytosterols.

5.3.11: Detection of Tannins

a) Gelatin Test - Test solution when treated with gelatin solution would give white precipitate indicating the presence of tannins.

5 ml each of the various extracts was dissolved in minimum amount of water separately filtered add filtrate were then subjected to the following test.

i) Ferric chloride Test- To the above filtrate a few drops of $FeCl_2$ solution were added. The colour change indicates the presence of tannins

ii) Basic lead acetate test- To the filtrate a few drops of aqueous basic lead acetate solution are added. Formation of reddish brown precipitate indicated the presence of tannins.

5.3.12: Detection of Xanthoprotein

1ml of various extracts was treated separately with a few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitation indicated the presence of xanthoprotein.

5.3.13: Detection of Terpenoids

An amount of 0.8 g of selected plant samples was taken in a test tube. Then poured 10 ml of ethanol in it, shaken well and filtered to take 5 ml extract. Then add 2 ml of chloroform were mixed in exact and 3 ml of sulphuric acid were added in the extract. Formation of reddish brown colour indicates the presence of Terpenoids.

5.3.14: Detection of Glycosides

a) Keller Killani Test - Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops 26 solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of conc. H_2SO_4 A brown ring at the interphase indicates the presence of cardiac glycosides.

b) Bromine water Test - Test solution was dissolved in bromine water and observed for the formation of yellow precipitates to show a positive result of the presence of glycosides.

5.3.15: Detection of Lignins

Phosphoglucinol with con.HCI was added with the test solution. Formation of pink colour indicates the presence of lignins.

5.3.16: Detection of Carotenoids

1g of each sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% H_2SO_4 was added. A blue colour at the interphase showed the presence of carotenoids.

5.3.17: Detection of Phlobatannins

Powdered plant sample was mixed with distilled water in a test tube. Then shake it well, and filtered to take plant extract. Then to each plant extract 1% aqueous hydrochloric acid was added and then boiled with the help of hot plate stirrer. Formation of red coloured precipitate confirmed a positive result.

5.3.18: Detection of Diterpenes

a) Copper acetate test - Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of Diterpenes.

5.3.19: Detection of Sugars

5 ml each of the various extracts was dissolved separately in distilled water filtered and then subjected to the following tests.

a) Molisch's Test- To the filtrate a few drops of alcoholic alpha- naphthol and 2 ml of conc. H_2SO_4 were added slowly through the sides of the test tube. Formation of reddish-brown precipitate indicated the presence of sugars.

b) Fehling's Test - A small portion of the various filtrate were treated with 1 ml of Fehling's solution 1 and 2 and then heated gently, change in colour indicated the presence of sugars.

5.4: Proximate Analysis of Ripe and Unripe *B. sapida* Aril

5.4.1: Determination of Moisture Content

The moisture content was determined according to AOAC method. The sample was taken in a flat bottom dish (pre-weighed); kept overnight in an oven at 100°C and weighed. The loss in weight was regarded as a measure of moisture content.

5.4.2: Determination of Crude fiber (Fibre – Muslin cloth method)

For determination of Crude fiber, 2 g material were treated with 200ml of 1 25% H2SO4 with 30 min boiling. After filtration and washing, the residue was treated with 1,25% NaOH with 30 min boiling, then filtered, washed with hot distilled water. The residue was dried overnight at 80-100 °C and weighed (m_1). It was then ignited and the ash weighed (m_2). Loss in the weight gives the weight of crude fiber calculated using the formula.

Crude fibre (%) = (100 x m_1- m_2)/ m_0, where m_1 is the weight of ashing dish with content before ashing, m_2 is the weight of ashing dish with content after ashing, m_0 is the weight of sample.

5.4.3: Estimation of Starch (Sedimentation method)

Starch content in aril was estimated by sedimentation method. A 5 gm sample was ground with 100 ml distilled water using a mortar and pestle. The mixture was separated through a cheese cloth and again added 50 ml distilled water. The filtrate was allowed to stand at overnight. After that starch was settled out. The filtrate was decanted off. So, the starch was left in the beaker. After that, 100 ml water added to rinse the starch. The process was repeated where the water was decanted off again. Lastly the wet starch was sundried to get a white powder. The percentage yield of isolated starches was determined using equation.

Starch%= ((Sample weight- final sample weight) / (Sample weight)) x 100.

5.5: Comparative study on the Nutritional content of the Ripe and Unripe *B. sapida* aril.

5.5.1: Estimation of Total Carbohydrate content (Hedge & Hofreiter, 1962)

The carbohydrate content was detected by Anthrone method. Take 1g of the sample into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCI and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence ceases. Made up the volume to 100 ml and centrifuged, collected the supernatant and take 0.5 ml for analysis. Prepared the standard by taking 0.2 -1.0 ml of the working standards, 1.0 ml of water serves as blank and made up the volume to 1.0 ml in all the tubes with distilled water, then added 4.0 ml of anthrone reagent, heated for eight minutes in a boiling water bath, cooled rapidly and read the green to dark green colour at 630nm.

5.5.2: Estimation of Protein by Lowry's Method (Lowry *et al*, 1951)

1g of the sample was weighed and ground well with a pestle and mortar in 5-10 ml of the buffer. Centrifuge it and used the supernatant for protein estimation. Pipetted out 0.2-1.0 ml of the working standard into a series of test tubes. Pipetted out 0.5 ml of the samples extracts in another test tube. Made up the volume to I ml in all the test tubes. A tube with 1 ml of water serves as the blank. Added 5 ml of reagent C (Alkaline copper solution) to each tube including the blank. Mixed well and allowed to stand for 10 min. Then added 0.5 ml of reagent D (Folin-Ciocalteau Reagent) mixed well and incubated at room temperature for 30 min. Blue colour was developed and read the colour at 660nm

5.5.3: Estimation of Fat (Cox and Pearson, 1962)

Dissolve sample in 50ml of the neutral solvent (25ml ether,25ml 95%alcohol) in a 250ml conical flask and few drops of phenolphthalein was added. This was titrated against 0.1N KOH until a pink colour which persists for 15seconds is obtained.

Fat Content = ((Titre Value x **Normality of KOH** x **56.1) / (Weight of the sample in gram))**

5.5.4: Determination of Nutritive value

The nutritional value of *B. sapida* aril was calculated as per the formula used by Nile and Khobragade (2009).

Nutritive value= (4 x percentage of protein) + (9 x percentage of fat) + (4 x percentage of carbohydrate).

5.6: Invitro Antioxidant Assay of Ripe and Unripe aril

5.6.1: Determination of Total Phenolic content (Slinkard & Singleton, 1977)

The total phenolic content of extracts was determined using the Folin-Ciocalteau's Phenol reagent. Pipetted out 0.5ml of sample into test tubes. Made up volume in each test tube to 3ml with distilled water. Add 0.5ml of Folin-Ciocaltueau reagent. After 3 minutes, added 2ml of 20% sodium carbonate solution to each test tube. Mixed thoroughly. Place the tubes in boiling water for exactly I minute. Cool and measure the absorbance at 650 nm against a reagent blank. The total phenol content was determined using the standard graph of pyrocatechol.

5.6.2: Determination of Flavonoid content (Boham and Kocipai, 1974)

2.5gm of aril sample was mixed with 80% of aqueous methanol and let it kept for 24 hrs. Discarded the supernatant, the residue re-extracted three times with same volume of methanol with Whitman filter paper. Sample filtrate was transferred to a pre-weighted Petri dish (**W_1**) and evaporated to dryness over a water bath. The content in the petri dish is cooled and weight of the dry petri dish (**W_2**) was recorded.

Total Flavonoid (%) = ((W_2 - W_1) / Sample weight) *100

5.6.3: DPPH Free Radical Scavenging Assay (Shimada *et.al* 1992).

The DPPH free radical scavenging assay was determined by the method of Shimada et.al (1992). 0.1mM DPPH (2,2-diphenyl -1-picrylhydarzil) was prepared in methanol solution. 0.5g of sample was homogenized using 5ml of methanol and centrifuged the contents. The supernatant was collected, different aliquots (0.5 and 1 ml) were prepared and final volume was made up to 1 ml using methanol. To this mixture added 2ml of 0.1mM DPPH solution (control) and reaction mixtures were measured at 517 nm against methanol as blank. The assay was carried out in triplicates. Lesser values of absorbance of the reaction mixture indicate

higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the formula.

DPPH Scavenged (%) = ((Absorbance of control – absorbance of test) ÷ (Absorbance of control)) × 100

5.6.4: Reducing Power Assay (Oyaizu,1986).

Antioxidant capacity as per reducing assay was measured according to a method reported by Oyaizu (1986). 1g of sample was extracted in 10ml phosphate buffer, contents were centrifuged and supernatant was collected for the assay. Volumes of 2.5ml of different concentrations of the extract were mixed with 2.5ml phosphate buffer solution (0.2M, pH = 6.6) and 2.5ml of 1% potassium ferric cyanide in test tubes. The mixtures were placed in water bath for 20 minutes at 50^0 C. After that 2.5 ml of 10% trichloro acetic acid (TCA) was added and mixed thoroughly. Then 2.5ml was taken from the mixture and added 2.5 ml of distilled water and 0.5ml of 0.1% $FeCl_2$ solution. The reaction mixture was allowed to stand for 10 minutes. Then the absorbance of the mixture was read at 700 nm against phosphate buffer as blank. The assay was carried out in triplicates. Higher values of absorbance of the reaction mixture indicate greater reducing power. Ascorbic acid standard was used as a positive control.

5.7: Antidiabetic activity of ripe and unripe aril

5.7.1: In vitro alpha amylase (PPA) Inhibition assay: Dinitrosalicylic acid (DNSA) method (Nirmali *et al*, 2016)

The inhibition assay was performed using the DNS method 1 ml of porcine pancreatic alpha amylase (PPA) enzyme solution (0.5 IU/ml) IN 0.02 M Phosphate buffer (ph. 6.9) was incubate with 1 ml of various plant extract for 20 minutes. The reaction was initiated by adding 250 microliters 0.5% soluble potato starch solution and the mixture was incubated for 15 minutes at room temperature. Then, 1 ml of colour reagent, DNS, was added and the mixture was placed in the boiling water bath. After 10 minutes the reaction mixture was diluted with distilled water and the absorbance was determined at 540 nm. Individual blanks were prepared for correcting the background absorbance. Controls were setup by replacing plant extract with 1ml of buffer. The result was expressed as percentage inhibition calculated using the formula

Percentage of inhibition activity= ((Absorbance of control – absorbance of test) ÷ (Absorbance of control)) × 100

5.8: Physicochemical analysis of *B. sapida* aril oil

5.8.1: Extraction of oil by Soxhlet

Oil was extracted from the aril using soxhlet apparatus and a heating mantle The apparatus is made up of a condenser, an extractor and flask. 30g of arils powder were packed in a thimble made from 4-fold cheese cloth. The thimble was loaded into the main chamber of the soxhlet extractor. For the extraction, petroleum ether (60°C) was used as the solvent and it was placed in the flask. When the solvent gets heated, it travels up a distillation unit and floods into the chamber housing the thimble of solid. The chamber containing the solid material slowly fills with warm solvent. The desired compounds dissolve in warm solvent. The solvent then returns to the distillation flask. This cycle is repeated many times over 6h. After continuous extraction, the oil was recovered in the distillation flask by evaporating the solvent by means of an oven. The thimble was pulled out and the final weight of the thimble was noted. The entire process was repeated thrice. The oil was collected in amber coloured bottles and stored in refrigerator. The total yield is expressed in percentage.

5.9: Chemical analysis of *B. sapida* aril oil

The extracted oil is subjected to following test to study the chemical properties of the *B. sapida* aril oil and compared with other edible oils like *sesame oil, bran oil, sunflower oil and coconut oil.*

5.9.1: Saponification Number (William Horowitz, 1975)

1g oil was weighed in a beaker and dissolved in about 3ml Fat solvent (Ethanol) and quantitatively transfer the content of the beaker 3 times with a further 7ml of the solvent. 25ml of 0.5N alcoholic KOH was added and mixed well. A blank was set with all other reagents present except the fat. Both the flasks were placed in a boiling water bath for 30 minutes, Cooled the flasks to room temperature. Then phenolphthalein indicator was added to both the flasks and titrated with 0.5N HCL The endpoint of blank and test was noted. The difference between the blank and test reading gives the number of millilitres of 0.5 N KOH required to saponify 1g of oil.

Saponification Value = ((28.05 x (Titre Value of Blank – Titre Value of sample)) / Weight of Sample in Gram)

5.9.2: Acid Value (Cox and Pearson, 1962)

Weighed accurately a quantity of the fatty oil (1g/5g) and placed it in a 250ml conical flask, then 50ml ethanol-ether solution was added and shake it well. Titrated the solution with sodium hydroxide titrant until pink coloration. The volume of sodium hydroxide titrant used was measured.

Free Fatty acid (FFA %) = ((Titre Volume x Normality of NaOH x 28.2) / (Weight of Sample in gram))

Acid Value = % FFA x 1.99

5.9.3: Peroxide Value (Cox and Pearson, 1962)

1g of oil was taken into a clean dry boiling tube and added Ig of powdered Potassium iodide and 20ml of solvent mixture. Placed the tube in boiling water so that the liquid boils within 30 seconds and allow to boil vigorously for not more than 30 seconds. Transfer the content quickly to a conical flask containing 20ml of Potassium iodide solution. Washed the tube twice with 25ml water each time and collect into the conical flask. Titrated against 0.5N Sodium thiosulphate solution until yellow colour is almost disappeared. Added 0.5ml of starch, shake vigorously and titrate carefully till the blue colour just disappears. A blank was also set at the same time.

Peroxide Value (milliequivalent peroxide/kg sample) = (S x N x 1000) / Weight of sample (gm)

Whereas S= (Test sample of $Na_2S_2O_3$ –Blank sample of $Na_2S_2O_3$) and N= Normality of $Na_2S_2O_3$

5.9.4: Iodine Number (William Horowitz, 1975)

Pipetted out 1 ml of oil sample dissolved in 1 ml chloroform to conical flask and labelled as "Test". Added 2ml of iodine solution in to the flask. Mixed the contents in the flask thoroughly. Then the flask was allowed to stand for thirty minutes' incubation in dark. Blank was set in another conical flask by adding 1 ml chloroform to the flask. Added to the blank, 2ml iodine solution and mixed the content in the flask thoroughly. Incubated the Blank in dark for 30 minutes. Test was taken from incubation after 30 minutes and 15%Potassium iodide solation was added in to the flask. Rinse the stopper and the sides of the flask using 50ml distilled water.

Titrated the Test against 0.5N sodium thiosulphate solution until a pale colour is observed, 1ml starch indicator was added in to the flask, a purple colour was observed. The titration was continued until the colour of the solution in the flask turns colourless. The disappearance of the blue colour was recorded as the end point of titration. The procedure is repeated for Blank.

Iodine Number = (S x N x 12.69) / Weight of sample (gm)

Whereas S= (Test sample of $Na_2S_2O_3$ –Blank sample of $Na_2S_2O_3$) and N= Normality of $Na_2S_2O_3$

5.10: Total Polar Compound by Column Chromatography

(Marquez-Ruiz *et al,*1996)

2.5 g of sample was weighted with the accuracy of 0.001 and diluted with the 20 mL of petroleum ether: diethyl ether solution (eluent solvent, 80:20 v/v). Samples were separated into triacylglycerols (nonpolar fraction) and polar fraction in glass column filled with silica gel and eluent solvent. Nonpolar fraction was eluted for 50 minutes with 150 mL of eluent mixture. After evaporation of the solvent the percentage of TPC was determined gravimetrically, the result was calculated according to the following equation

TPC (%) = ((ms-mn)/ms) *100

TPC – Total polar compounds, ms– Initial sample mass, mn – Nonpolar fraction mass

5.11: Assay of Lipase Enzyme

Lipase hydrolyses triglycerides to release free fatty acids and glycerol

$$\text{Triglycerides} + H_20 \longrightarrow \text{Glycerol} + \text{Fatty Acids}$$

Substrate and enzyme source is needed for the assay of lipase enzyme. For the preparation of substrate, take 10ml of clear vegetable oil and stirred with 9ml of distilled water in the presence of 25mg bile salt till an emulsion is formed. For the preparation of enzyme source, grinded 1gm of sesame seeds with a mortar and pestle and homogenized the tissue with a medium containing 0.25M sucrose and 10mM Tris HCl at pH 7and filtered through cheese cloth. Assay mixture containing 5ml vegetable oil emulsion, 5ml Tris buffer and 1ml crude enzyme.

Incubated the mixture containing at 35^0C for 10 minutes. The reaction was stopped by adding 10ml of acetone: methanol mixture in the ratio 1:1.

Each sample was titrated against 0.02N NaOH using 1%Phenolphthalin as indicator. An appearance of light pink colour indicates the end point. The volume of NaOH used in titration was noted and it is used for determining the enzyme required to liberate 1mM of the free fatty acid from the oil per minute under the Standard assay condition. Specific activity is expressed as 1mMol/1min/g of protein.

Specific activity of lipase enzyme (meq/min/g sample) = ((Volume of alkali consumed x Strength of alkali) / (Weight of sample in gram x Time in minute))

Figures

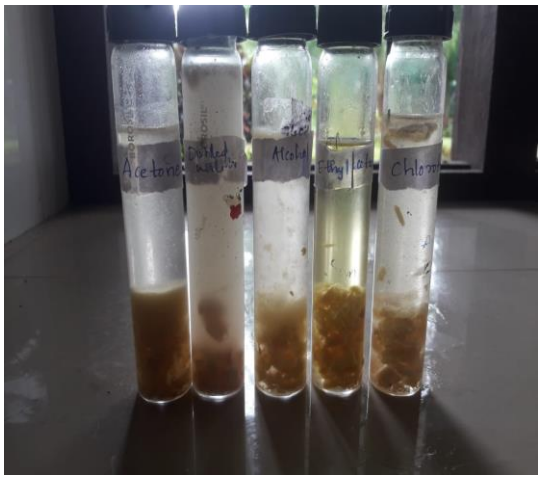

Fig 5.3: Aril Extract

Fig 5.4: Aril for drying

Fig 5.3: Aril Flour

Fig 5.4: Soxhlet Apparatus

Fig 5.5: Unripe aril

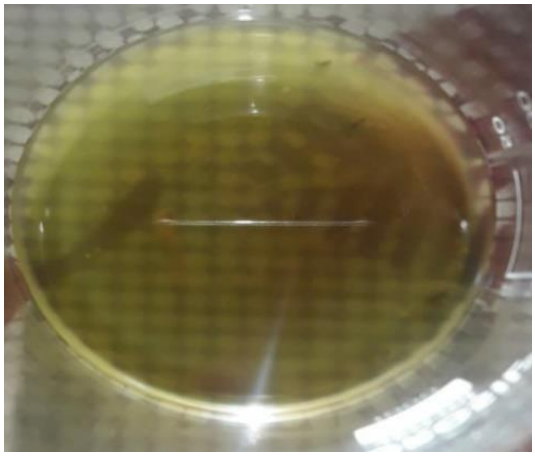

Fig 5.6: Oil Extracted from Soxhlet

Column chromatography for analysis of Total polar materials

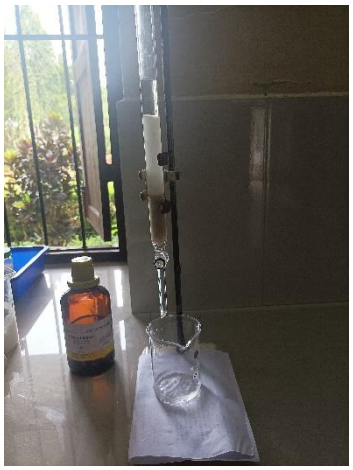

Fig 5.7: Sesame Oil

Fig 5.8: *B. sapida* Oil

Fig 5.9: Bran Oil

Fig 5.10: Sunflower Oil

Fig 5.11: Coconut Oil

6. RESULT AND DISCUSSION

The proximate composition, the nutritive value and antioxidant potential of *B. sapida* aril and its oil was done by the methods mentioned above.

6.1: Preliminary Phytochemical analysis

The results of phytochemical screening of *B. sapida* aril indicated the presence of some secondary metabolites that may be responsible for the bioactivity. Table 6.1 shows the phytochemical activity.

Table 6.1: Preliminary Phytochemical analysis

Phytochemical Constituents	Solvents Used				
	Ethyl Acetate	**Chloroform**	**Distilled Water**	**Ethanol**	**Acetone**
Flavonoids	-	-	+	+	+
Alkaloids	+	-	+	+	-
Glycosides	+	+	-	-	-
Phytosterol	-	+	-	+	+
Phenol	-	-	+	+	+
Tannin	-	-	+	+	+
Terpenoids	-	-	-	-	+
Carotenoids	-	-	-	-	+
Carboxylic acid	-	-	+	+	-
Xanthoprotein	-	-	+	-	+

Resins	-	-	+	+	+
Coumarins	+	+	-	-	+
Quinones	+	-	+	-	+
Protein	-	-	+	+	+
Sugar	-	-	+	-	+
Diterpenes	+	+	-	-	+
Saponins	+	+	-	-	-
Phlobatannins	-	-	-	+	-

The Table 6.1 shows that the Ethyl acetate extract reveals the presence of Alkaloids, Glycosides, Coumarins, Quinones, Diterpenes and Saponins. Chloroform reveals the presence of glycosides, Phytosterols, Coumarins, Diterpenes and Saponins. The distilled water extract reveals that the presence of flavonoids, Alkaloids, Phenols, Tannins, Carboxylic acid, Xanthoprotein, Quinones, Proteins and Sugars. The Ethanol extract contains the presence of Flavonoids, Alkaloids, Phytosterols, Phenols, Tannins, Carboxylic acid, Resins and Proteins. Acetone extract reveals the presence of Flavonoid, Phytosterols, Phenols, Tannins, Terpenoids, Carotenoids, Xanthoprotein, Resins, Coumarins, Quinones, Proteins, Sugars and Diterpenes. From the study, it was observed that the acetone extract and the distilled water extract of *B. sapida* aril showed more different classes of phytochemicals than in other extracts.

6.2: Analysis of the Proximate Composition of *B. sapida* unripe and ripe Aril

6.2.1: Determination of Moisture Content

The moisture content of *B. sapida* aril was found to be 42% moisture in unripe aril and ripe *B. sapida* aril contains 72% moisture (Table 6.2). Veronica *et al*, (2014) reported that the aril contained 48.3 to 52.20% of moisture. The moisture content of *B. sapida* aril was variable according to the maturity of fruit, climate and other physical factors.

6.2.2: Determination of Crude fiber

The quantification of Crude fiber was done to find out the nutritional and medicinal importance of the *B. sapida* aril. The result was expressed as a measure of percentage (%) in the sample.

The crude fibre content of *B. sapida* aril was found to be 2.2% in unripe aril and 3.7% in ripe aril (Table 6.2). Veronica *et al*, (2014) reported that the aril contained 3.63 to 3.82% of crude fibre. The high fibre content in ripe aril serves as a good source of dietary fibre which plays a very important role in lowering the blood cholesterol levels.

Dietary fiber or roughage is the portion of plant-derived food that cannot be completely broken down by human digestive enzymes. Dietary fibers are diverse in chemical composition, and can be grouped generally by their solubility, viscosity, and fermentability, which affect how fibres are processed in the body. Dietary fiber has two main components: soluble fiber and insoluble fiber, which are components of plant foods, such as legumes, whole grains and cereals, vegetables, fruits, and nuts or seeds. A diet high in regular fiber consumption is generally associated with supporting health and lowering the risk of several diseases.

6.2.3: Determination of Starch

Starch is the main storage polysaccharide of plants and it is the most important dietary source for human beings. The starch content in unripe aril and ripe aril was found to be 12.3% and 21.7% respectively (Table 6.2). On a dry weight basis, the seeds contained 43.3% starch (Abiodun *et al*, 2015).

Starch or amylum is a polymeric carbohydrate consisting of numerous glucose units joined by glycosidic bonds. This polysaccharide is produced by most green plants for energy storage. Worldwide, it is the most common carbohydrate in human diets, and it is contained in staple foods such as wheat, potatoes, maize (corn), rice, and cassava (manioc).

Table 6.2: Proximate composition of *B. sapida* unripe and ripe Aril

Sl No	Proximate Analysis	Unripe Aril(%)	Ripe Aril(%)
1	**Moisture Content**	**52**	**72**
2	**Crude Fibre**	**2.2**	**3.7**
3	**Starch**	**12.3**	**21.7**

6.3: Analysis of Nutritional content of Unripe and Ripe Aril

6.3.1: Determination of Carbohydrate

Carbohydrates, also known as saccharides or carbs, provide energy for the body. Each gram of carbohydrates provides 4 calories. The body breaks carbohydrates down into glucose, which is the primary energy source for the brain and muscles. Carbohydrates are one of three macronutrients, which are nutrients that the body needs in larger amounts. The carbohydrates are important for healthy diet. The result revealed that the presence of carbohydrates in the unripe aril was 2.34mg/g (Fig 6.1) and the ripe aril was 6.62mg/g (Fig 6.1). The study conducted by Anupama and Sunil Kumar (2019) reported that the aril contains 10.31mg/g carbohydrate. The carbohydrate content of *B. sapida* aril was variable according to the maturity of fruit, climate and other physical factors and also the variation may be due to the inability of the absorption of nutrients from the soil.

6.3.2: Determination of Protein

The protein content was determined by Lowry's method and the result was found to be 1.8mg/g in unripe aril (Fig 6.1) and 4.13mg/g in ripe aril (Fig 6.1). Anupama and Sunil Kumar (2019) reported that the aril contains 0.415mg/g protein.

Most microorganisms and plants can biosynthesize all 20 amino acids, while animals (including humans) must obtain some of the amino acids from the diet. The amino acids that an organism cannot synthesize on its own are referred to as essential amino acids. Key enzymes that synthesize certain amino acids are not present in animals such as aspartokinase, which catalyses the first step in the synthesis of lysine, methionine, and threonine from aspartate. If amino acids are present in the environment, microorganisms can conserve energy by taking up the amino acids from their surroundings and downregulating their biosynthetic pathways. Protein helps in the synthesis and growth of muscles and tissues.

6.3.3: Determination of Fat

Most of the fat found in food is in the form of triglycerides, cholesterol, and phospholipids. Some dietary fat is necessary to facilitate absorption of fat-soluble vitamins (A, D, E, and K) and carotenoids. Humans and other mammals have a dietary requirement for certain essential fatty acids, such as linoleic acid (an omega-6 fatty acid) and alpha-linolenic acid (an omega-3

fatty acid) because they cannot be synthesized from simple precursors in the diet. Both of these fatty acids are 18-carbon polyunsaturated fatty acids differing in the number and position of the double bonds. Most vegetable oils are rich in linoleic acid (safflower, sunflower, and corn oils). Alpha-linolenic acid is found in the green leaves of plants and in some seeds, nuts, and legumes (in particular flax, rapeseed, walnut, and soy). Fish oils are particularly rich in the longer-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Many studies have shown positive health benefits associated with consumption of omega-3 fatty acids on infant development, cancer, cardiovascular diseases, and various mental illnesses (such as depression, attention-deficit hyperactivity disorder, and dementia).

In contrast, it is now well-established that consumption of trans fats, such as those present in partially hydrogenated vegetable oils, are a risk factor for cardiovascular disease. Fats that are good for one may be turned into trans fats by improper cooking methods that result in overcooking the lipids.

The Fat content in Unripe and ripe aril was found to be 6.70mg/g and 9.81mg/g respectively (Fig 6.1). Anupama and Sunil Kumar (2019) reported 4.7mg/g lipid in aril.

6.3.4: Determination of Nutritive Value

By Nile and Khobragade formula (2009) the nutritive value estimated for unripe aril is 120.6 K Cal/100g and 148.4 K Cal/100g for ripe aril. From this we can understand that the ripe *B. sapida* aril is nutritionally promising because of the greater amount in the biomolecules like Carbohydrate, Protein and lipids.

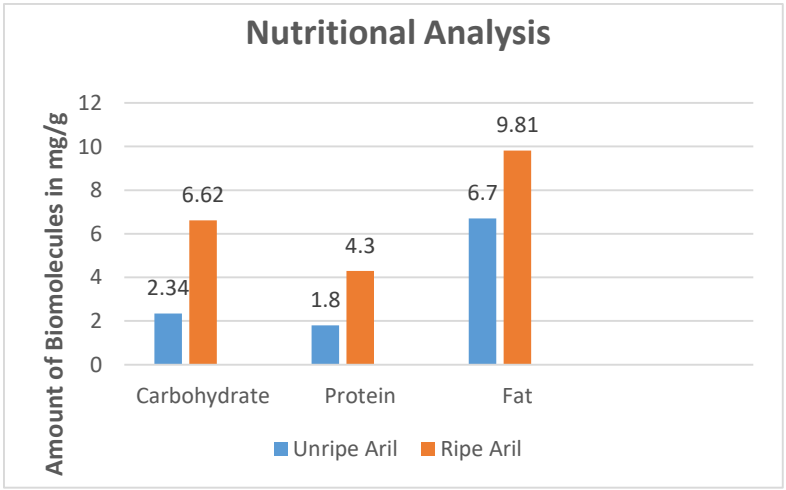

Fig 6.1: Nutritional Analysis of Unripe aril and Ripe aril.

6.4: In vitro Antioxidant Assay of Ripe and Unripe Aril of *B. sapida*

6.4.1: Determination of Total Phenolic content

Phenolic compounds are known as powerful chain breaking antioxidants, which may contribute directly to antioxidative action. These compounds are very important constituents of plants and their radical scavenging ability is due to their hydroxyl groups. In human phenolic compounds have been reported to exhibit a wide range of biological activities including antibacterial, anti-inflammatory and antioxidant property. The phenol content of unripe *B. sapida* aril was found to be 4.25 mg/g (Table 6.3) and 5.22 mg/g for ripe aril (Table 6.3). The study conducted by Emmanuel *et al,* in 2014 reported that the seed contains 317.20±0.89 mg/100g phenol content.

Table 6.3: Total Phenol Content of Aril

Aril	Total Phenol Content
Unripe aril	**4.25mg/g**
Ripe aril	**5.22mg/g**

6.4.2: Determination of Flavonoid content

The biological function of flavonoids includes protection against allergies, inflammation, free radical's platelet aggregation, microbes, ulcers, hepatoxins, viruses etc. The total flavonoid content of the unripe aril was estimated as1.5% (Table 6.4) and in the ripe aril was estimated as 2.8% (Table 6.4). The study conducted by *Anupama* and *Sunil Kumar* in 2019 reported that the ripe aril contains 1.3% flavonoid content which is slight variant to the current study. The flavonoid content of *B. sapida* aril was variable according to the maturity of fruit, climate and other physical factors.

Table 6.4: Total Flavonoid Content of Aril

Aril	Total Flavonoid Content
Unripe aril	**1.5%**
Ripe aril	**2.8%**

6.4.3: Determination of in vitro Antioxidant activity by DPPH Method

The antioxidant activity of *B. sapida* aril was determined using methanol solution of DPPH (Shimada *et.al*, 1992). The result suggested that the unripe aril contain 33% (Fig 6.2) and the ripe aril contain 24% antioxidant activity (Fig 6.2). Veronica *et al*, (2014) reported the antioxidant activity in the ripe aril were 29.4 % DPPH inhibition which is slight variant to the current study. From the current study it was found that the antioxidant activity is more in unripe aril than ripe aril of *B. sapida*.

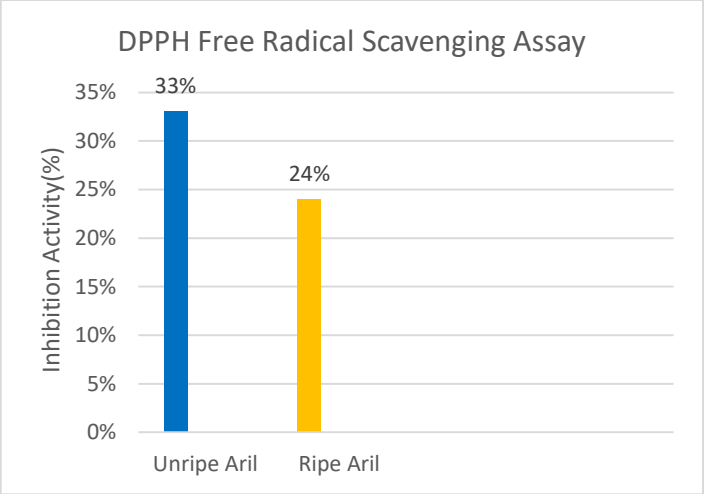

Fig 6.2: Invitro antioxidant potential of ripe and unripe aril

6.4.5: Determination of Reducing Power Assay

The reducing Power assay was carried down in the aril of *B. sapida*. Higher values of absorbance of the reaction mixture indicate greater reducing power. From the figure 6.3, the ripe aril has highest reducing power than Unripe aril.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reductive ability was measured in terms of Fe^{3+} to Fe^{2+} transformation in the presence of different concentrations of the extract. The presence of reductants in extracts causes the reduction of Potassium hexacyanoferrate (K_3FeCN_6) to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the absorbance, where it is directly proportional to the reducing power of test substance. Antioxidant action of the Reductones is based on the breaking of free radical's chain by the donation of a hydrogen atom. Reductones are believed not only to react directly with peroxides but also to prevent peroxide formation by reacting with certain precursors (Ilhami *et al*, 2007).

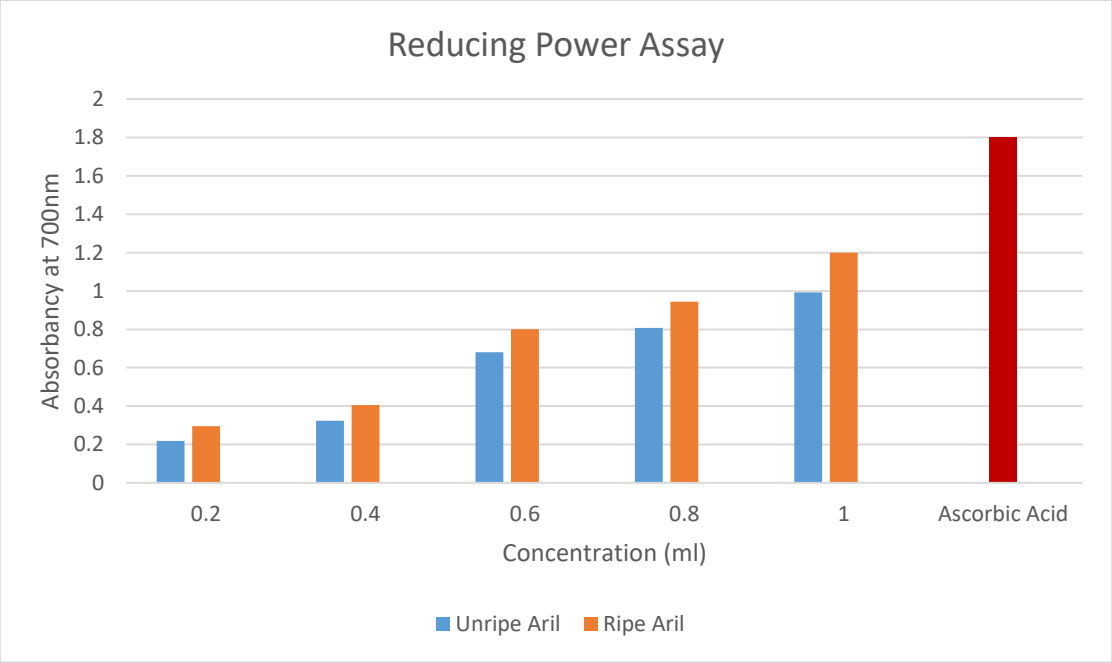

Figure 6.3: - Reducing Power Assay of Ripe and Unripe Aril

6.5: Antidiabetic activity of Ripe and Unripe aril

6.5.1: Determination of alpha amylase (PPA) Inhibition

Diabetes has caused a major burden to the health sector in the developing countries and has shown an increasing trend among the urban population. It is estimated that most patients are with type II diabetes which could be easily treated with dietary changes, exercise, and medication. The α-amylase inhibition assay was performed using the 3,5- dinitrosalicylic acid method showed that the *B. sapida* unripe aril contain 75% inhibition (Table 6.4) and the ripe aril contain 26% inhibition (Table 6.4). From this result, it was found that unripe aril shows antidiabetic activity.

Table 6.4: Alpha amylase Inhibition of Aril

Aril	Alpha amylase Inhibition
Unripe aril	**75%**
Ripe aril	**26%**

The antidiabetic effect is due to the antioxidant properties of the aril (Rajesh *et al*, 2011; Mahendran *et al*, 2011). Plants having hypoglycaemic effect are known to be rich in phenolic compounds, coumarins, flavonoids, terpenoids and other constituents which reduce blood glucose levels (Jung *et al*, 2006).

Analysis of *B. sapida* aril oil

6.6: Oil content in the sample

Petroleum ether solvent was used for the extraction of oil. After the 6 hours of continuous oil extraction at 60^0 C using soxhlet apparatus, oil was collected in amber coloured bottles and stored. The oil yield was 60%. According to Tsado *et al,* (2018), the aril had the oil yield of 47.05±0.54 % which was observed to be slightly lower.

6.7: Chemical analysis of Oil

The chemical properties of oil are among the most important properties that determines the condition of oil. Chemical characteristics of oils like acid value, peroxide value, saponification number and iodine number of the aril oil were done.

The result obtained from acid value and saponification value was 30.86% and 715.2 mg KOH/g of oil respectively (Fig 6.4 and 6.5). Tsado *et al,* (2018) reported free fatty acid value is in the range of 4.91±0.16 to 9.02±0.34 mg KOH/g of oil and saponification value is in the range of 175.23±2.52 to 193.73±1.85 mg KOH/g of oil. Elizabeth *et al,* (2011) reported free fatty acid value were 1.83 (±0.01) mg KOH/g of oil and saponification value were 743 mg per 100g of oil. In fatty acids, unsaturation occurs mainly as double bonds which are very reactive towards halogens, the iodine in the case of determining iodine value of oils. Thus, the iodine number of the *B. sapida* oil was found to be 14.50 mgI_2/g (Fig 6.7). Tsado *et al,* (2018) reported iodine value is in the range of 63.72±2.43 to 116.54±1.00 mgI_2/g of oil.

A product with peroxide value between 1 and 5 mgeq/kg has low oxidation state. Oils with high peroxide values are unstable and easily become rancid. The peroxide value from the aril was estimated as 1100 mgeq/kg (Fig 6.6). Tsado *et al,* (2018) reported peroxide value is in the range of 5.05±0.21 to 9.44±0.09 mEq/k and Elizabeth *et al,* (2011) reported iodine value were 28.01 mEq/kg.

When comparing the *B. sapida* aril oil with other edible oils, the acid value and saponification number (Fig 6.4 and 6.5) and the iodine number (Fig 6.7) are in a similar range. But a slight variation could be seen in the case of peroxide value (Fig 6.6) when compared with the other edible oil.

Figures: Chemical Characters of Individual Oil

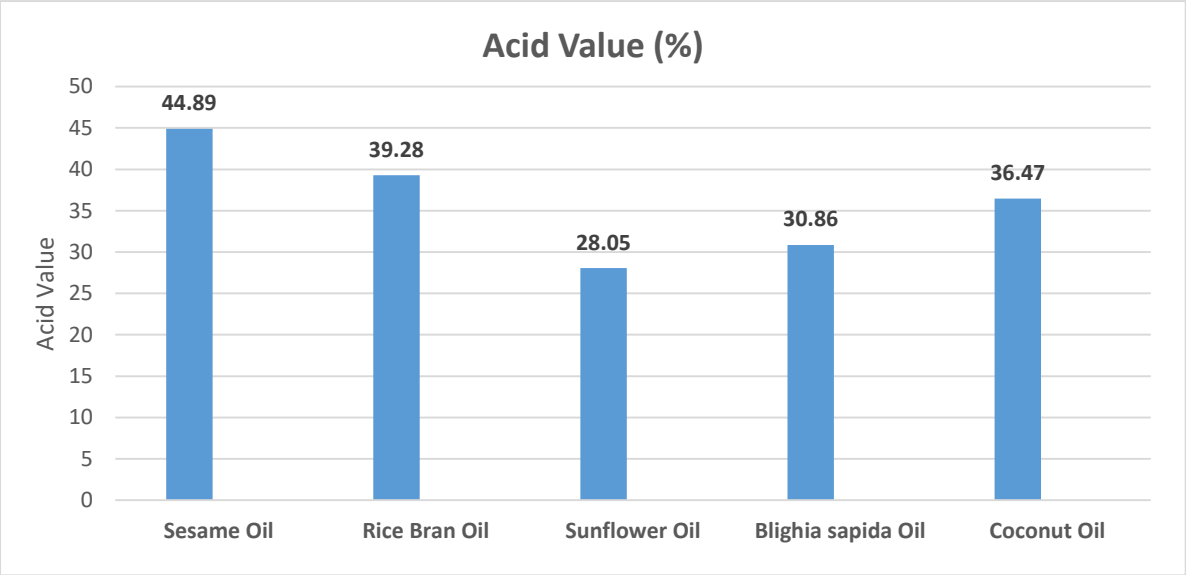

Fig 6.4: Acid value

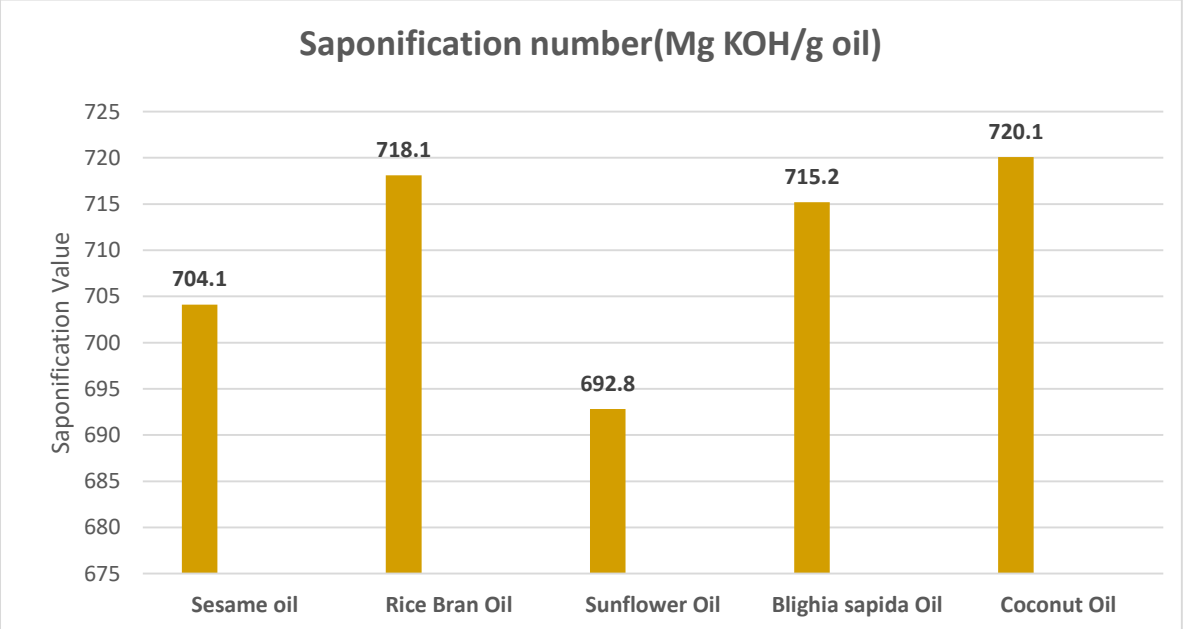

Fig 6.5: Saponification number

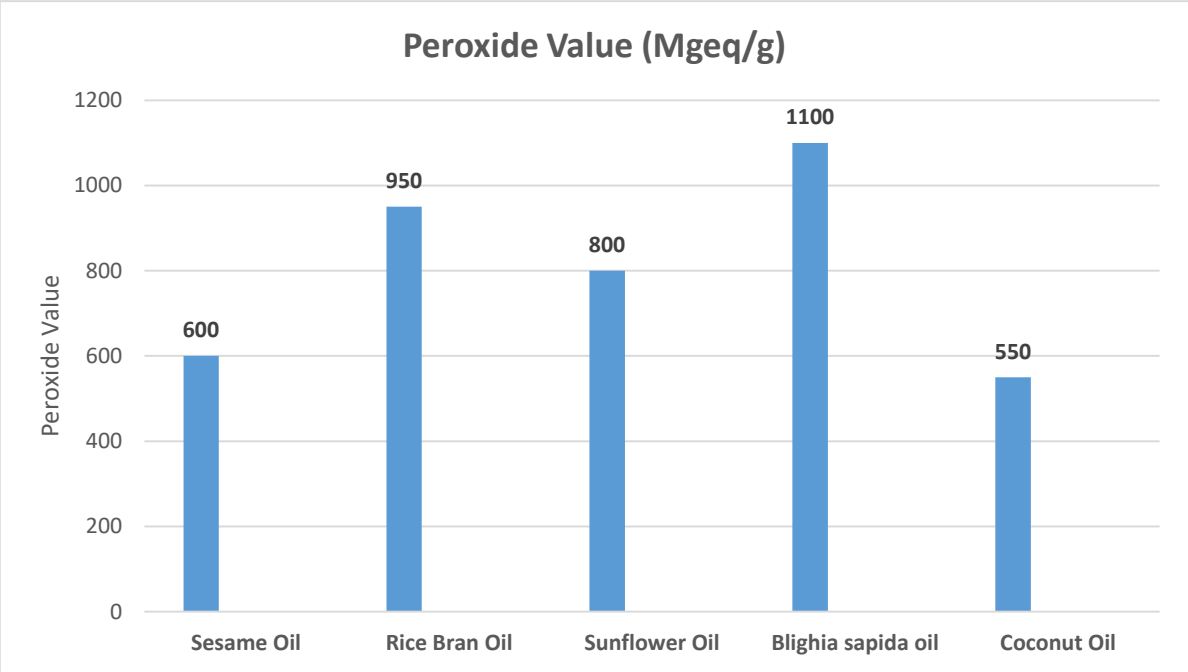

Fig 6.6: Peroxide value

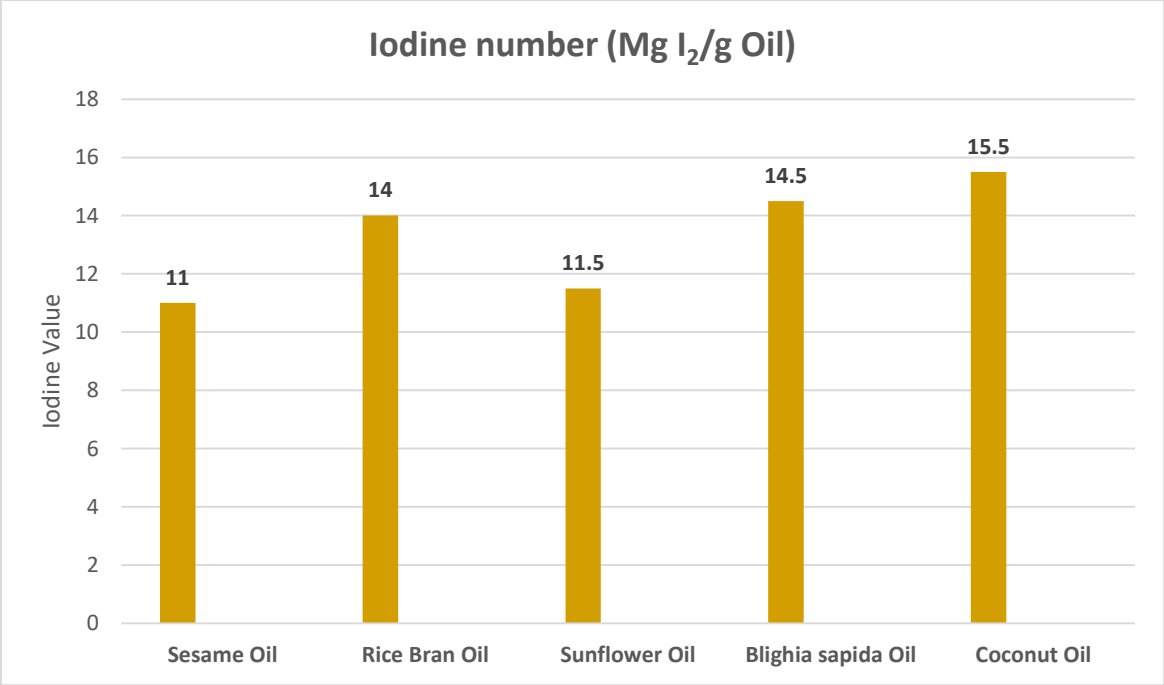

Fig 6.7: Iodine value

6.8: Estimation of Total Polar Material (TPM)

Column chromatography was done to obtain the polar fractions from the extracted oil and TPM % was calculated.

Total polar compounds in fats and oils are variable. Various factors are responsible for the formation of polar compounds in the frying oil. The Total polar material is considered to be a chemical index of oil degradation. At the 3rd International symposium on frying in 2000, the international community established that 24% TPM is the percentage at which frying oil is no longer suitable for human consumption.

The result obtained from the analysis of TPM shows that the *B. sapida* oil is suitable for frying process (Figure 6.8) and the oil show almost similarities when compared with *sunflower oil, coconut oil, rice bran oil, sesame oil.*

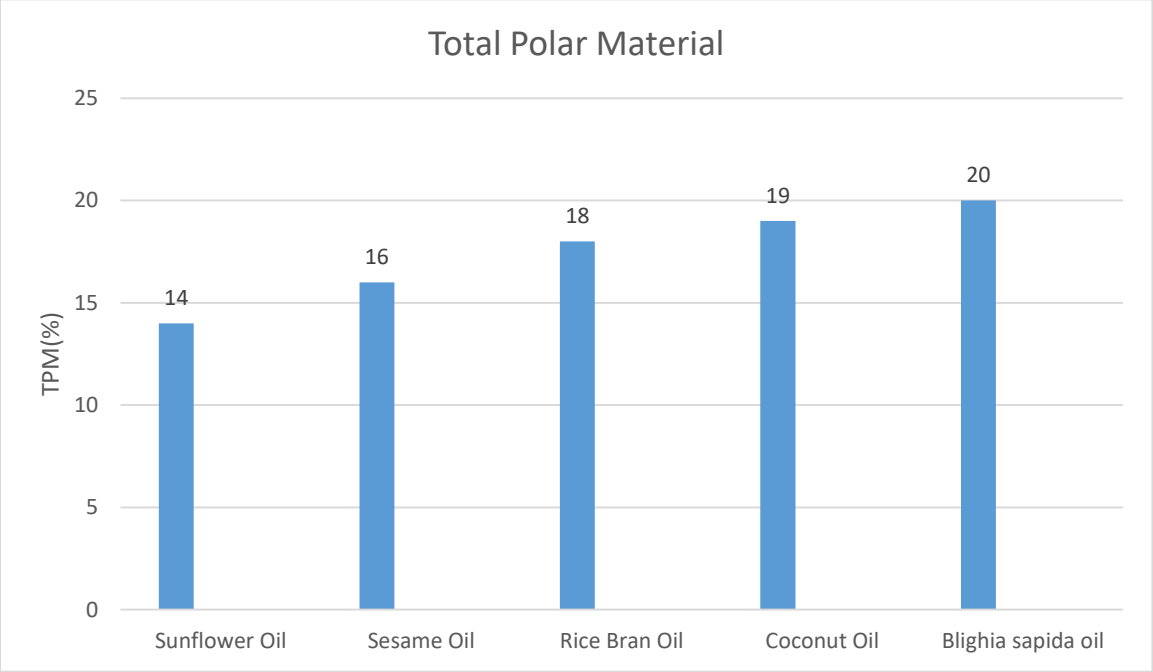

Fig 6.8: Total Polar Material

6.9: Assay of lipase Enzyme

During germination of oil seeds, lipases play an important role in hydrolysing the stored oil so that the required energy for growth and carbon skeleton for synthesis of new compounds are produced. Germinating seeds of coconut, sunflower, sesame and groundnut are good source of lipase enzyme. (Jayaraman, 1981)

From the figure 6.9, the lipase activity of *B. sapida* oil is 84% which is closely related to the lipase activity of Sunflower oil. So, *B. sapida* oil can be used for the synthesis of fine chemicals and pharmaceuticals, manufacture of shampoo, soap, paper etc. the oil can also be used for flavour development in dairy products and processing of other food containing fats

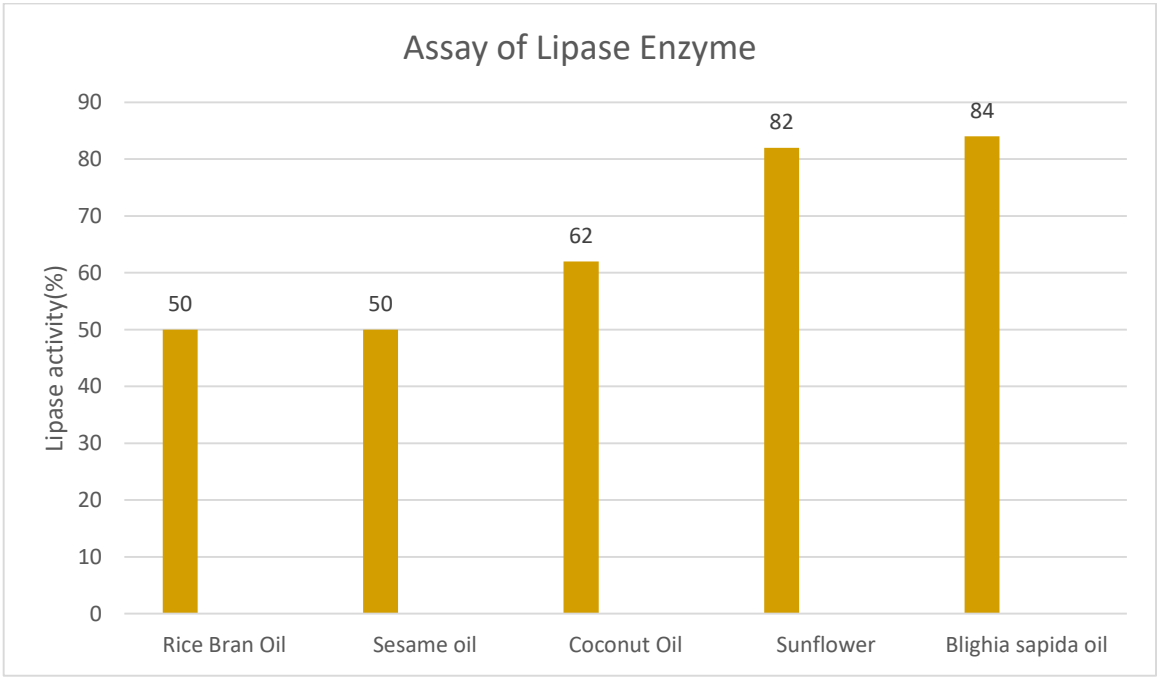

Fig 6.9: Assay of lipase Enzyme

7. SUMMARY AND CONCLUSION

Blighia sapida fruit has comparatively less importance in all over the world except Jamaica. The edible portion of this fruit is somehow not used because of the toxic substance present in the aril called hypoglycin A. This study focused on the proximate composition, phytochemicals and antioxidant properties of the aril and also the physicochemical properties of oil obtained from the edible aril.

Phytochemical screening of *B. sapida* revealed the presence of flavonoids, alkaloids, glycosides phytosterol, phenol, tannin, terpenoids, carotenoids, carboxylic acid, xanthoprotein, resins, coumarins, quinones, protein, sugar, diterpenes, saponins and Phlobatannins.

The quantification of moisture content, crude fibre and starch were estimated as a part of proximate analysis. The comparative analysis based on the nutritional quality of ripe and unripe arils of *B. sapida* showed that the carbohydrate, protein and fat were rich in both arils. The nutritional value for unripe aril and ripe aril is 120.6 K Cal/100g and 148.4 K Cal/100g respectively. So, it can be concluded that the ripe *B. sapida* aril is nutritionally promising because of the presence of more amount of nutrients.

The phenol content of unripe and ripe aril was found to be 4.25 mg/g and 5.22 mg/g respectively. The total flavonoid content was also found to be higher in ripe aril (2.8%). Bioactive Polyphenols and Flavonoids have attracted special attention because they can protect the human body from the oxidative stress which may cause many diseases, including cancer, cardiovascular problems and ageing.

The present study indicated the presence of antioxidants in the *B. sapida* aril. The result obtained from antioxidant assay using DPPH method showed that the unripe aril of *B. sapida* has a better antioxidant property than the ripe aril. The reducing power assay showed a high absorbancy in the ripe aril extract which denote a significant reducing power.

Antidiabetic α-amylase inhibition assay using the 3,5- dinitrosalicylic acid method showed that the *B. sapida* ripe aril contains less inhibition than in the unripe aril. It was found that unripe aril has an anti-hyperglycaemic effect.

Average oil yield obtained from the ripe aril of *B. sapida* was 60% which indicates an elevated level of lipid content in ripe aril. Chemical properties such as acid value, saponification

number, iodine number and peroxide value were analysed with other edible oils commonly used in Kerala such as *coconut oil, rice bran oil, sunflower oil and sesame oil.*

The acid value and saponification number of *B. sapida* aril oil was almost similar to other edible oil and showed the low acidity so that the oil is free from hydrolytic rancidity. The saponification number is also similar to the edible oil and the high saponification number indicates that the *B. sapida* aril oil can be used for the manufacture of shampoos, soaps, creams etc. The iodine number is also similar to the edible oil, among them it is very similar to coconut oil, so that the *B. sapida* oil is highly saturated and good for the manufacture of soaps. But the *B. sapida* oil shows high peroxide value when compared with other edible oils, so this indicates that the oil has a chance for the production of peroxide during storage conditions.

At the 3rd International symposium on frying in 2000, the international community established that 24% total polar content (TPM) is the percentage at which frying oil is no longer suitable for human consumption. The result obtained from the analysis of TPM shows that the *B. sapida* oil is suitable for the frying process. Due to the high lipase enzyme activity, the oil can be used for the synthesis of fine chemicals and pharmaceuticals, manufacture of shampoo, soap, paper etc. The oil can also be used for flavour development in dairy products and processing of other food containing fats. Based on the above findings, it could be concluded that *B. sapida* oil and the ripe arils could be used as a potential source for the functional ingredients. The current study also showed that unripe aril possesses antidiabetic property. Further studies are needed to identify the active compounds responsible for the hypoglycaemic effect.

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