Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Studies on antioxidant activities of areca nut (*Areca catechu* L) extracts and their fractions

Suresh D

Dept. of Chemistry, UCS, Tumkur University, BH Road, Tumkur-572 103, Karnataka, India
Email: pbdsuresh@gmail.com, Contact No: +91 9886465964

ABSTRACT

The Areca nut is well known for its traditional use across the world from ancient times. The current study involves extraction of the areca nut powder with various solvents for effective extraction of bioactive compounds. These extracts were evaluated for the levels of polyphenols and flavonoids contents, study the antioxidant potential of the extracts, fractionate the most active extract and assess the antioxidant activity of the fractions. It is observed that methanol extract is proved to have highest flavonoids content followed by water, ethanol-water & ethyl acetate. Ethanol-water extract was found to have highest percent of polyphenols about 2.6% followed by methanol, water and ethyl acetate extracts. It was observed that, methanol extract is shown to exhibit potent antioxidant activity followed by ethyl acetate extract, ethanol-water extract and water extract. Due to regulatory limitations, methanol extract could not be considered for fractionation further. The ethanol-water extract was fractionated and found that fraction – 4 was found to be highly potent.The studies are important from the stand point of the potential antioxidant activities of the extracts and their fractions.

KEY WORDS: *Areca nut, antioxidant activity, extracts, flavonoids, polyphenols, fractions.*

***Correspondence Author**

Dr. Suresh D

Dept. of Chemistry, UCS, Tumkur University,

Tumkur, Karnataka, India, PIN – 572103. Fax: +91–8162260220-

E-Mail: pbdsuresh@gmail.com

1.0 INTRODUCTION

The Areca nut is the seed of the Areca palm (*Areca catechu*), which grows in much of the tropical Pacific, Asia, and parts of east Africa. It is commonly referred to as "betel nut" as it is often chewed wrapped in betel leaves. Areca nuts are chewed with betel leaf for their effects as a mild stimulant, causing a mild hot sensation in the body and slightly heightened alertness, although the effects vary from person to person. The effect of chewing betel and the nut is relatively mild and could be compared to drinking a cup of coffee. In almost all parts of India, Sri Lanka and southern China areca nuts are not only chewed along with betel leaf but are also used in the preparation of Ayurvedic and Traditional Chinese medicines. Powdered areca nut is used as a constituent in some tooth powders. Other medicinal uses include the removal of tapeworms and other intestinal parasites by swallowing a few teaspoons of powdered areca nut, drunk as a decoction, or by taking tablets containing the extracted alkaloids.

Betel nuts have been used as a drug for thousands of years. The practice is thought to have started in south-east Asia and there is archaeological evidence to support this view. The Spirit Cave site in Thailand yielded palaeobotanical remains of Areca catechu and Piper betel, traditional consumption is a combination of Areca catechu, Piper betel, and edible lime, since found at the same location, it is circumstantial evidence for the practice of betel chewing in prehistoric times.

Antioxidant compounds in food play an important role as a health protecting factor. It is evident that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical

scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature by Miller and Rigelhof etal.[1,2]

Antioxidants are widely used as ingredients in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation is harmful. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation[3]. These compounds may be synthesized in the body or obtained from the diet[4]. The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors.

Antioxidants are used as food additives to help guard against food deterioration. Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. However, as oxygen is also important for plant respiration, storing plant materials in anaerobic conditions produces unpleasant flavors and unappealing colors[5]. Consequently, packaging of fresh fruits and vegetables contains an ~8% oxygen atmosphere. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food[6]. These preservatives include natural antioxidants such as ascorbic acid and tocopherols, as well as synthetic antioxidants such as propyl gallate, tertiary butylhydroquinone, butylated hydroxyanisole and butylated hydroxytoluene[7].

The most common molecules attacked by oxidation are unsaturated fats; oxidation causes them to turn rancid[8]. Since oxidized lipids are often discolored and usually have unpleasant tastes such as metallic or sulfurous flavors, it is important to avoid oxidation in fat-rich foods. Thus, these foods are rarely preserved by drying; instead, they are preserved by smoking, salting or fermenting. Even less fatty foods such as fruits are sprayed with sulfurous antioxidants prior to air drying. Oxidation is often catalyzed by metals, which is why fats such as butter should never be wrapped in aluminium foil or kept in metal containers. Some fatty foods such as olive oil are partially protected

from oxidation by their natural content of antioxidants, but remain sensitive to photooxidation[9]. Antioxidant preservatives are also added to fat-based cosmetics such as lipstick and moisturizers to prevent rancidity.

Antioxidants are frequently added to industrial products. A common use is as stabilizers in fuels and lubricants to prevent oxidation, and in gasolines to prevent the polymerization that leads to the formation of engine-fouling residues[10]. In 2007, the worldwide market for industrial antioxidants had a total volume of around 0.88 million tons. This created revenue of 3.7 billion US-dollars.

Natural antioxidants such as α-tocopherol and L-ascorbic acid are widely used because they are seen as being safer and causing fewer adverse reactions, but their antioxidant activities are, however, lower than those of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Hence, the need exists for safe, economic antioxidants with high activity from natural sources to replace these synthetic chemicals. The antioxidant compounds present in edible plants have recently been promoted as food additives because they display little or no toxic side effects.

The number of antioxidant compounds synthesized by plants as secondary products, mainly phenolics, serving in plant defence mechanisms to counteract ROS in order to survive, is currently estimated to be between 4000 and 6000[11]. A direct relationship has been found between the content of total phenolics and antioxidant capacity of plants[12]. In fact, to counteract deleterious action of ROS, phenolic compounds, naturally distributed in plants, are effective[13]. Because purified phenolic compounds are difficult to obtain and because extracts sometimes have better antioxidant activities than those of pure molecules, there is a growing interest for the use of plant extracts[14]. To find new natural sources of active compounds, we studied the antioxidant potential of different extracts of Areca catectu L. The use of betel nut, as a masticatory by humans has been known since the 4th century A. D. in different parts of the world. It is estimated that over 600 million individuals consume areca nut (also called betel nut) in one form or another world-wide. In old Indian scripts, such as Vagbhata (4th century), and Bhavamista (13th century), betel nut has been described as a therapeutic agent. Its use was recommended in many diseases, such as leucoderma, leprosy, anaemia, and obesity. It was also reported to have deworming properties. In China, it has been used as a vermifuge since the 6th century and is still employed as such in some parts[15]. In the Philippines the flowers are sometimes added to salads. The nuts, husks, young shoots, buds, leaves, and roots are used in various medicinal preparations[16].

Although it has already been demonstrated that areca fruit contain total phenolics and tannin[17], little is known about the antioxidant potential of areca fruit, areca extracts and their fractions. Accordingly, the current investigation examined the antioxidant properties of areca

extracts and fractions of methanolic extracts through biochemical assays of DPPH (1, 1 – diphenyl – 2 -picrylhydrazyl) and the polyphenols and flavonoids content were quantified. The investigation involves extraction of the areca nut powder with solvents of varied polarities for effective extraction of bioactive compounds, assess the levels of polyphenols and flavonoids content in the extracts, study the antioxidant potential of the extracts, fractionate the most active extract and assess the antioxidant activity of the fractions.

2.0 MATERIALS & METHODS

1, 1 - Diphenyl – 2 - picrylhydrazyl was procured from Sigma-Aldrich India Company. Ascorbic acid, Gallic acid, Vanillin, Phloroglucinol and Methanol were purchased from S. D. Fine Chemicals. All other solvents are of AR grade and distilled before use. Distilled water was employed for all the experiments. Areca nut was collected in the month of August – September in Sira taluk of Tumkur district, Karnataka. The sample were shade dried and powdered into 100 mesh size and was stored at room temperature in a airtight container until extraction.

2.1 Preparation of extracts:

Hot water extract preparation: Hot water soluble polar compounds can be extracted by this method. In this case, cold water insoluble compounds but soluble in hot water can be extracted. 1: 10 proportion of material to solvent was taken for extraction and the extraction is carried out at boiling temperature of water (100^0 C) with a reflux arrangement for 3 hours with constant stirring and the extract is filtered and centrifuged to remove any un-dissolved material. The extract is then concentrated to 1/5 volume on the concentrator and dried completely. Thus prepared extract is stored in airtight bottles.

80% Ethanol extract preparation: Ethanol-water soluble polar compounds can be extracted by this method while the proteins and polysaccharides get precipitated. Here too, 1: 10 proportion of material to solvent was taken for extraction and the extraction is carried out at boiling temperature of ethanol (65^0 C) with a reflux arrangement for 3 hours with constant stirring and the extract is filtered and centrifuged to remove any un-dissolved material. The extract is then concentrated to dryness. Thus prepared extract is stored in airtight bottles.

Ethanol extract preparation: Ethanol soluble polar compounds can be extracted by this method while the proteins and polysaccharides get precipitated. Here too, 1: 10 proportion of material to solvent was taken for extraction and the extraction is carried out at boiling temperature of Ethanol with a reflux arrangement for 3 hours with constant stirring and the extract is filtered and

centrifuged to remove any un-dissolved material. The extract is then concentrated to dryness. Thus prepared extract is stored in airtight bottles.

Methanol extracts preparation: Methanol soluble polar compounds can be extracted by this method while the proteins and polysaccharides get precipitated. Here too, 1: 10 proportion of material to solvent was taken for extraction and the extraction is carried out at boiling temperature of Methanol with a reflux arrangement for 3 hours with constant stirring and the extract is filtered and centrifuged to remove any un-dissolved material. The extract is then concentrated to dryness. Thus prepared extract is stored in airtight bottles.

2.2 Polyphenol assay:

Folin ciocalteu reagent (0.1N) was prepared by diluting1:20 with commercially available FC Reagent with distilled water to get the required concentration. Sodium carbonate (7.5%) was prepared by dissolving 7.5 gm of sodium carbonate in 100ml of de-ionized water. Gallic acid (standard) stock I (Conc. 0.1 mg/ml) was prepared by dissolving 1mg of gallic acid in 10 ml with 50% Methanol. For making standard graph of Gallic acid concentration range of 2 - 20 µg/ml was used. The assay was carried out by Using Singleton method[18]. In brief, to a 200 µl of 50% Methanol / Standard / test sample with various concentrations, added 1000 µl of FC reagent, mixed and incubated at RT for 5min. added 800µl of 7.5% sodium carbonate, mixed and incubated at RT for 30 minutes. Read the absorbance at 750 nm against blank by spectrophotometer, Color correction was given with the same concentration of the test sample in 50% Methanol without FC reagent.

2.3 Flavonoids assay:

Vanillin Reagent (1%) was prepared by dissolving 1gm of crystallized vanillin in 100 ml of 70% Conc. H_2SO_4 (Prepared fresh). Conc. H_2SO_4 (70%) was prepared bydiluting 70 ml on Conc. H_2SO_4 in 100ml De-ionized water. Methanol (50%) was prepared by diluting 1:1 with de-ionized water. Phloroglucinol (standard) stock I (Conc. 1mg/ml): Dissolved 10mg of Phloroglucinol and made up to a volume of 10 ml with 50% Methanol, Then centrifuge at 12,000 rpm for10min. Stock II: Diluted to a conc. to yield 0.1mg/ml with 50% Methanol. For making standard graph of Phloroglucinol, 1 – 10 µg/ml concentration range was used. The Flavonoid assay was carried out by using Swain&Hillis method[19].In brief, to a 400µl of distilled water / Positive control / test sample with various concentrations, added 800µl of 1% vanillin reagent, mixed and incubated at RT for 15 minutes. Read the absorbance at 500 nm against blank by spectrophotometer. Color correction was given with the same concentration of the test sample in distilled water without vanillin reagent. The

Flavonoid content in the phytoextracts was measured with reference to the standard Gallic acid values.

2.4DPPH assay:

Dissolved 39.4mg of DPPH in 100ml of methanol to get concentration of 1mM stock. Stored in dark bottle at 4°C until its use. The working concentration of DPPH in the assay was 0.14mM. Methanol (50%) was prepared by diluting methanol 1:1 with de-ionized water. Ascorbic acid standard Stock I (Conc. 200µg/ml) was prepared by dissolving 2 mg of ascorbic acid and make up to a volume of 10ml with de-ionized water. For making standard graph of ascorbic acid 2, 4, 6, 8, 10µg/ml concentration range was used. The DPPH assay was carried out by using modified method of Brand-Williams[20], in brief to a 860µl of 50% methanol / ascorbic acid / test sample with various concentrations, added 140µl of 1mM DPPH, mixed and incubated at 37^{o} C for 30min. Read the absorbance at 520 nm against 50% methanol blank by spectrophotometer, a control reaction is carried out by without test sample addition. Colour Correction contains the same concentration of the test sample in methanol without DPPH. The anti-oxidant activity was measured with reference to the standard ascorbic acid absorbance values. The actual absorbance is taken as the absorbance difference of the control and the test sample and IC_{50} values were determined.

2.5Fractionation:

The column was prepared using silica gel of 60 to 120 mesh size. The sample was dissolved with 5 ml of the solvent and sample bed was made with impregnation at the top.The column was elutedwith mobile phase 4:1acetone and ethyl acetateand collected the fraction - 1. This is followed by collection of two of the fractions with the mobile phase composition of 1:1 and 1:4 acetone and ethyl acetate. Another fraction was collected by eluting with 100% ethanol.

3.0 RESULTS & DISCUSSION

Free radicals were a major interest for early physicists and radiologists and much later found to be a product of normal metabolism. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. As a consequence, reactive oxygen species are known to be implicated in many cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, cataracts, chronic inflammation, and neurodegenerative diseases. ROS and free radicals are also considered as inducers of lipid peroxidation and cause the deterioration of foods. Although organisms have endogenous antioxidant defences produced during normal cell aerobic

respiration against ROS, other antioxidants are taken from the diet, both from natural and synthetic origin.

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death. When the chain reaction occurs in a purified monomer, it produces a polymer resin, such as a plastic, a synthetic fiber, or an oil paint film. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.

As oxidative stress appears to be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease. Antioxidants are widely used as ingredients in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation is harmful. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.

Antioxidants, which can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very important in the prevention of many diseases. Thus, synthetic antioxidants are widely used in the food industry. However, because of their toxic and carcinogenic effects, their use is being restricted. Thereby, interest in finding natural antioxidants, without undesirable side effects, has increased greatly.

Because purified phenolic compounds are difficult to obtain and because extracts sometimes have better antioxidant activities than those of pure molecules, there is a growing interest for the use of plant extracts. To find new natural sources of active compounds, we studied the antioxidant potential of different extracts of *Areca catechu.*

Extraction Yields

The solvents such as water, Ethanol-water, Ethyl acetate and Methanol were utilized to optimize extraction process so as to arrive at extract with higher yields and better antioxidant potency. It was observed that Methanol is the solvent which provides highest yield among all the solvents. Water with ethanol were also selected as the extraction solvents since both are commonly used in the food industry in a variety of ways, and are more highly stable in the human body than any other solvents. The extraction yield is high because a low extraction yield means a lower productivity despite high antioxidation. The extraction yields were expressed in terms of the solid content in the dried product per soluble solid content in areca nut used on a dry basis. Table 1 shows the extraction yields of the various extracts from areca nut.

Sl. No.	Solvent of Extraction	% Flavonoid Contents
1	Water	26.5 ± 0.28
2	Ethanol – Water (80:20)	21.5 ± 0.47
3	Ethyl acetate	6.0 ± 0.38
4	Methanol	30.5 ± 0.16

Table – 1: The percentage yield of the extracts with various solvents.

SD - Standard Deviation. Values are average of three independent extractions

Despite the low values obtained for the extraction yields, the antioxidant contents found were good, indicating that the extraction was efficient. Nevertheless, a relationship between the extracted mass and the corresponding total phenolics and flavonoids were observed in all cases. Most of the phenolic or polyphenolic compounds in nature have antioxidant activities, e.g. tocopherols, flavonoids and derivatives of cinnamic acid, phosphatidic and other organic acids.

Polyphenol Contents

Polyphenol content was determined by Singleton method. It was found that the ethanol-water extract was found to have highest percent of polyphenols about 2.6% followed by methanol, water and ethyl acetateextracts (table-2). From the results it can be concluded that ethanol-water extract

Table – 2: The polyphenol contents of various extracts

Sl. No.	Solvent of Extraction	% Yield
1.	Water	8.66 ± 1.15
2.	Ethanol – Water (80:20)	9.6 ± 1.11
3.	Ethyl acetate	5.72 ± 2.01
4.	Methanol	13.4 ± 1.87

Table – 3: The Flavonoid content of various extracts.

Sl. No.	Solvent of Extraction	% Polyphenol Contents
1	Water	1.2 ± 0.1
2	Ethanol – Water (80:20)	2.6 ± 0.2
3	Ethyl acetate	1.05 ± 0.1
4	Methanol	1.4 ± 0.1

SD - Standard Deviation. Values are average of three independent determinations

could be highly potential in terms of health beneficial physiological effects followed by the trend explained as above.

Flavonoids Contents

It is evident from above data that methanol extract is proved to have highest flavonoid content (table-3). This is followed by water, ethanol-water & ethyl acetate.

Sl. No.	Solvent of Extraction	IC_{50} Values (µg/ml)
1	Water	380 ± 10.2
2	Ethanol – Water (80:20)	370 ± 8.6
3	Ethyl acetate	300 ± 5.3
4	Methanol	280 ± 8.1

Table – 4: The DPPH activity of various extracts

SD - Standard Deviation. Values are average of three independent determinations

The methanol extract could be potent extract in terms of flavonoid contents which could elicit very useful health beneficial activities.

DPPH Activity

DPPH·, a stable free radical with a characteristic absorption at 517 – 520 nm, was used to study the radical scavenging effects of ethanol extracts. The decrease in absorption is taken as a measure of the extent of radical scavenging. The radical-scavenging activity (RSA) values were expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH· solution in the absence of extract at 520 nm. From the analysis of, we can conclude that the scavenging effects of all extracts on DPPH radicals increased with the concentration increase and were excellent, especially in the case of methanol extract. The RSA value was also good for ethyl acetate extract.It is observed that, methanol extract is shown to exhibit potent antioxidant activity having IC_{50} value of 280 µg/ml followed by ethyl acetate extract, ethanol-water extract and water extract (table-4).

Sl. No.	Fraction	IC_{50} Values (µg/ml)
1	4:1 acetone and ethyl acetate (fraction – 1)	130 ± 1.86
2	1:1 acetone and ethyl acetate (fraction – 2)	86 ± 2.58
3	1:4 acetone and ethyl acetate (fraction – 3)	118 ± 2.79
4	100% Ethanol (fraction – 4)	71 ± 3.78

Table – 5: The DPPH activity of various fractions

SD - Standard Deviation. Values are average of three independent determinations

Additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their potent bioactive properties and the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods. This explains why no single antioxidant can replace the combination of natural phytochemicals to achieve the health benefits. Analysis of the results revealed that antioxidant activity increased with the concentration, good results being obtained, even at low extract concentrations.

It is evident from above data that methanol extract is proved to have highest contents of flavonoid content. This is followed by water, ethanol-water & ethyl acetate. The methanol extract could be potent extract in terms of flavonoid contents which could elicit very useful health beneficial activities.

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

A considerable body of literature supports a role for oxidative stress in the pathogenesis of age-related human diseases and a contribution of dietary antioxidants to their prevention. The complex relationships between antioxidant status and disease are still poorly understood and have

been studied intensively. For many years, polyphenols and other antioxidants were thought to protect cell constituents against oxidative damage through scavenging of free radicals. However, this concept now appears to be an oversimplified view of their mode of action. More likely, cells respond to antioxidants mainly through direct interactions with receptors or enzymes involved in signal transduction, which may result in modification of the redox status of the cell and may trigger a series of redox-dependent reactions. Both antioxidant and prooxidant effects have been described, with contrasting effects on cell physiologic processes.

Antioxidants may improve cell survival; as prooxidants, they may induce apoptosis and prevent tumor growth. However, the biological effects of antioxidants may extend well beyond the modulation of oxidative stress. One of the best-known examples involves the interaction of soy is o flavones with estrogen receptors and the effects of these compounds on endocrine function. These effects could explain the prevention by is of lavones of bone resorption among postmenopausal women. A detailed understanding of the molecular events underlying these various biological effects is essential for evaluation of the overall impact on disease risk and progression.

Unequivocally, methanol extract emerges out as the best possible extract in terms of the yield as well as antioxidant activity. However, methanol is not a suitable solvent for its consumption as it causes severe deleterious effects even at very low concentrations. Due to this, the regulatory bodies all across the globe have very stringent norms for using methanol as solvent of extraction. In lieu of these facts, the comparatively potent ethanol-water and water extract could be utilized as potential antioxidant extracts. In terms of yield, phytochemical contents and activity, ethanol-water extract proves to be the best extract. Hence, the same was further subjected for fractionation by using column chromatography.

Among the four fractions collected, fraction – 4 (100% ethanol) was shown to exhibit highly potent activity having IC_{50} value 71µg/ml(Table – 5)followed by fraction – 2, fraction – 3 and fraction – 4.

4.0 CONCLUSIONS

The present investigation deals with the extraction, separation, phytochemical constituent analysis and antioxidant activity analysis. It was observed that the yields of extraction depend on nature of solvent. It was found that the methanol is the best possible solvent for having higher yields compared to other solvents chosen of varied polarities.

Polyphenol content determination indicates that the ethanol-water extract was found to have highest percent of polyphenols about 2.6% followed by methanol extract. However, the other

extracts also contain considerable amounts of the polyphenols. This indicates that all these extracts may have significant antioxidant activities thereby eliciting beneficial physiological effects.

The Flavonoid estimation of the extracts indicate that methanol extract possess highest amount of flavonoids among all the extracts of different solvents. Other extracts also have substantial amounts of flavonoids. Since the flavonoid content is directly proportional to the antioxidant activity, these extract could exhibit potential antioxidant activities.

The DPPH activity analysis indicates that methanol extract evidenced to have potential antioxidant activity among all extracts of different solvents. This is probably due to the highest flavonoid content compared to other extract. It is also clear that areca nut seems to have highly soluble compounds in methanol; hence the yield is also high.

In view of the regulatory constraints, methanolic extract could not be considered for the fractionation. The ethanol-water extract was fractionated and found that fraction – 4 (100% ethanol fraction) was found to be highly potent. Further fractionation may yield highly potent molecules which could be used as effective antioxidant molecules.

5.0 REFERENCES:

1. Miller HE, Rigelh of F, Marquart L, et al. Whole-grain products and antioxidants. Cer. Foods World. 2000; 45: 59-63.
2. Miller HE, Rigelh of F, Marquart L, et al. Antioxidant content of whole grain breakfast cereals, fruits and vegetables. J. Am. Coll. Nutri. 2000; 19: 312S-319S.
3. Sies H, Oxidative stress: Oxidants and antioxidants. Exper. physiol. 1997; 82: 291–95.
4. Vertuani S, Angusti A, Manfredini S, The Antioxidants and Pro-Antioxidants Network: An Overview. Curr. Pharm. Design. 2004; 10: 1677–94.
5. KaderA, ZagoryD, KerbelE, Modified atmosphere packaging of fruits and vegetables. Crit. Rev. Food Sci. Nutr. 1989; 28: 1–30.
6. ZallenE, HitchcockM, GoertzG, Chilled food systems. Effects of chilled holding on quality of beef loaves. J. Am. Diet. Assoc.1975; 67: 552–57.
7. IversonF, Phenolic antioxidants: Health Protection Branch studies on butylated hydroxyanisole. Cancer Lett. 1995; 93: 49–54.
8. RobardsK, Kerr A,PatsalidesE. Rancidity and its measurement in Edible oils and snack foods, Analyst 1988; 113: 213–24.
9. Del CarloM, SacchettiG, Di MattiaC, et al. Contribution of the phenolic fraction to the antioxidant activity and oxidative stability of olive oil. J. Agri. Food Chem. 2004; 52: 4072–79.

10. BoozerE, Charles, HammondS, et al. Air Oxidation of Hydrocarbons. II. The Stoichiometry and Fate of Inhibitors in Benzene and Chlorobenzene. J. Am. Chem. Soc.1955; 77: 3233–7.
11. HavsteenBH, The biochemistry and medical significance of the flavonoids. Pharmacol. Therapeut.2002; 96: 67–202.
12. WollgastJ, Anklam, Review on polyphenols in Theobroma cacao: changes in composition during the manufacture of chocolate and methodology for identification and quantification. Food Res. Int.2000; 33: 423–47
13. PereiraJA, Pereira APG, FerreiraICFR, et al. Table olives from Portugal:Phenolic compounds, antioxidant potential, and antimicrobial activity. J. Agri. Food Chem. 2006;54: 8425–31
14. CallisteCA, TrouillasP, AllaisDP, et al. Leaves as new sources of natural antioxidant: an electron spin resonance study. J. Agri. Food Chem. 2005; 53: 282–8.
15. SharanRN. Association of betel nut with carcinogenesis: a review. Cancer J.1996; 9: 1.
16. StaplesGW, BevacquaRF. Species Profiles for Pacific Island Agroforestry:Areca catechu. (areca nut palm), August, 2006
17. ZhangCJ, LvFJ, TaiJX, et al. Quantitative determination of total phenolics and tannin in areca nut and its products. Food Res. Dev. 2008;29:119-21.
18. SingletonVL, Rossi JrJA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. 1965;16:144-58.
19. SwainT. HillisWE. The phenolic constituents of Purmus domestica. I. The quantitative analysis of phenolic constituents. J. Sci. Food Agri. 1959; 10: 63.
20. Brand-WilliamsW. Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. (London) 1995; 28: 25–30.