CHAPTER 2

LITERATURE REVIEWS

2.1 Betel Nut (*Areca catechu* L.)

Betel nut (*A. catechu*) is belonging to the Palmaceae family, distributed much in the Tropical Pacific, Asia, and parts of East Africa (Staples, G.W. & Bevacqua, R.F., 2006). Betel quid (mixture of areca seed, lime, and piper betel) chewing culture is presented for a long time in Asia. In India and other Southern Asian areas, the seed of betel nut which can be obtained from its fruit is used as one component in betel quid. In Taiwan, fresh-unripe betel nut fruit is the main ingredient of betel quid.

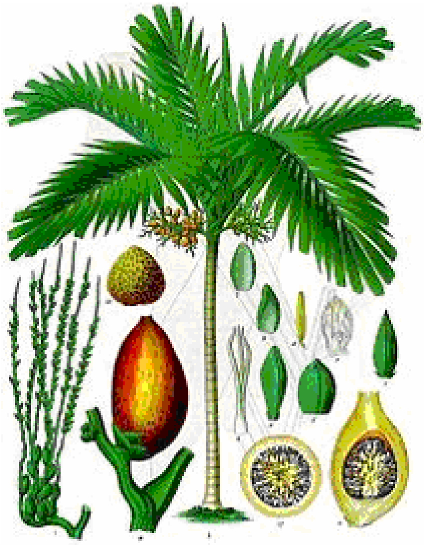

Figure 2.1 Betel Palm Morphology

Tree: Betel nut is a slender, single-stemmed palm tree, up to 30 m high. It has a crown of 8-12 leaves at the top.

Stem: cylindrical stem and about 10-15 cm wide. It is covered with rings, which are the scars of old leaves that have died and dropped off. The flower sheaths were used as a packing material and can also be used to make dishes, small umbrellas or even skull caps. The trunk often formed the roof rafters of poorer houses in Bombay, and wood from the palm was put walls of houses. The hollow stems make a good water channel. The palm wood has sometimes been used to make small items like boxes or pen trays (Swarup). Pieces of the stems of betel nut palms are used as brush handles to apply pigment in Indian mural paintings. Betel nut stem is preferred for this as the wood does not react with the color of the pigments.

Leaves: 1 to 1.5 m long and feather-shaped. The base of each leaf completely encircles the palm stem like a tube. The leaves and leaf sheath can be used as a source of fiber basketry and containers. The leaves and petioles the betel nut palm are woven to make cups, bags and plates for food and water. They can also be written on and are useful for making fans, umbrellas and hats.

Flowers: both male and female flowers appear on the same plant on branched inflorescences below the crown of leaves. Male flowers grow close to the tips of flowering branches, while female flowers grow close to the base. They are tiny, fragrant and creamy-white in color.

Palm hearts: As with most palms, the tender shoot of the palm stem, known as the cabbage or palm heart, is edible. In India it is cooked in syrup.

Fruits: ranging in color from yellow through orange to red, 5cm in diameter and oval. There is one seed (called the nut) inside the fruit and it varies in shape from oval to round. The palm is cultivated for the hard, dried inner layer (endosperm) of its seeds. This is chewed by people in South Asia as a stimulant. The fruits are used as a source of tannin for dyeing.

Seed: The green unripe fruits were used to tan leather in South India. The fruits contain tannin and can be boiled down into a solid brown mass called 'catechu'. Catechu is used as an orange-brown dye and is also used as a source of tannin for preparing fibers before using other dyes.

Figure 2.2 Betel Nut Palms Cultivated in South Asia

Betel pepper: Leaves from the betel pepper plant, *Piper bete*l L. are used a 'wrapping' for betel quid. It is a climbing plant with shiny, green, heart-shaped leaves. It is only known in cultivation but is thought to have originated in Malaysia, Sumatra and possibly Java. Today it is cultivated in all betel chewing areas. The leaves are known as paan in India, and plant is known as tambuli in Hindi.

Betel paan: The most basic betel chew, or paan, consists of the sliced nut or supari, wrapped with slivers of lime (calcium hydroxide) in leaves of the betel pepper vine, together with some catechu (a vegetable extract from an acacia tree). For a more elaborate chew other aromatic condiments would be included. For a stronger effect, a twist of tobacco is frequently utilized in a plain chew.

Figure 2.3 Cultivation of *Piper betel* L. (Betel Pepper Leaf)

All the ingredients combine to stain the saliva red and give a red tinge to the lips and mouth. It has an energizing effect and is often used to stave off tiredness. It has also become part of a ritual symbolizing gracious living. Betel nuts play an important role in south Asia. The fresh or dried endosperm of seeds of the betel nut palm is chewed alone or in combination with a leaf of betel pepper (*Piper betel* L.), lime paste and catechu, which is a scarlet and astringent extract made from boiling chips of wood of the betel nut palm.

Other ingredients may be added to the little package, known as a betel quid, including tobacco, palm sugar and various spices such as cardamom (*Elettaria cardamomum*) and clove (*Syzygium aromaticum*). The rich assortment of paraphernalia involved in making up betel mixtures include receptacles for each of the separate ingredients, mortars, dishes, spittoons and cutters for the betel nuts. The mixture is also taken after meals to sweeten the breath.

Chewing the quid increases the production of saliva, which is blood red in color, and gastric juices and so is believed to aid digestion. After chewing, the quid is spat out. There can be up to thirteen ingredients, including camphor, copra, pepper, betel nut, cardamom, cloves, musk, lime and catechu.

Figure 2.4 Sliced Betel Nut (Supari)

Figure 2.5 Betel Quid Composed of Calcium Hydroxide (Slaked Lime), Betel Pepper Leaf, Sliced Betel Seed and Additional with Tobacco

Spiritual and religion: In parts of India, the offering and chewing of betel nuts fulfills an important religious and social function. It is a symbol of well-being and represents a refined lifestyle. Betel nut forms an integral part of many Indian rituals and ceremonies. Here, paan is being prepared for a wedding. The seeds of betel nut palms are so important that they form an integral part of most rituals from birth to death in Indian society. Offerings of betel nuts are made at social occasions as a lucky token for all relationships. Betel nut is offered to images of gods as part of religious ceremonies. In India betel nut seed chewing is a symbol of the Hindu Trinity, and betel nut seeds are believed to delight Brahma. Sometimes the flowers of the betel nut palm are used in ceremonies such as weddings.

Figure 2.6 Betel Quid Fulfill Indian Ceremony Atmosphere

History: Betel nut palms originally grew wild in Southeast Asia, perhaps in the Philippines or Malaysia. They were brought to the Indian subcontinent by humans in prehistoric times. The betel nut palm is now cultivated across the Asian tropics as a cash crop, as well as in almost every village garden. It is used for its seeds which are chewed as a stimulant.

South Asian history: Betel nut's origins in India cannot be precisely dated but it has been mentioned in ancient literature and texts for almost 2,000 years. In a Tamil classic story, Silappadikaram, the heroine feeds her husband with betel chews. Betel nuts also feature in ancient traditional medicine. Ayurvedic medical texts dating back 2000 years discuss the ingredients of betel quids, and Sanskrit writings list different types of betel quid. More precise references to betel nut chewing were dated from the Gupta period in the 5th century AD. By this time, it appears to have been a widespread practice.

Betel nut chewing is cited as one of eight enjoyments, or bhogas, of royal life in the 11th century text called *Manasollasa* or Splendour of Thought. It was mentioned by Marco Polo in the 13th century, and the 14th century traveler Ibn Battuta wrote of how it was part of all palace meals in the Delhi Sultanate. One of the earliest surviving Indian cookbooks, dating from about 1500, was produced for the Sultan of Mandu. It shows him supervising the preparation of elaborate betel nut paan. In the 15th century, a Persian ambassador called Abdul Razzak visited the south of India and wrote about the invigorating effects of betel nut chewing. By the 17th century, Indian writings noted the best palm varieties to use, and where they were grown. As betel nut chewing evolved, so have the accessories used to make betel quids. They include fine boxes and seed cutters.

Origins: The betel nut palm is only known in cultivation, and its exact origin is not known. It is likely to have evolved in South East Asia where it is thought to be of very ancient cultivation and where diversity of the genus *Areca* is greatest. The chewing of the seeds is an ancient tradition for millions of people across South and Southeast Asia. The practice is known as 'tamboul' in India and may have originated in the region of Vietnam and Malaysia. From here it was probably first introduced to India.

Production and trade: The betel nut palm is widely grown in South Asia, particularly India, Pakistan and Bangladesh, for its stimulating properties. India is the most important betel nut palm growing country in the world. The production of betel nuts has increased from 75,000 tons in 1955 to 330,000 tons in 2003. Betel nut cultivation in India now covers some 716,605 acres.

In Thailand, the main betel nut cultivation areas (Table 2.1) were in the southern of Thailand, Chum-phorn, Nakorn Sri Thammarat, Ranong, Pattalung, Trang, Pang-nga, and Surat Thani, respectively. The eastern of Thailand also cultivate betel palm in Cha Chaerng Sao, Rayong, and Nakorn Prathom, respectively (Thai Department of Agriculture Extension, 2002). The betel palm also widely cultivated in all part of Thailand such as Prae in the northern. The latest report of Thai Ministry of Commerce report 2012 betel nut export value was 1,153.3 million baht which more than 35% raised from 2011 (Thai Ministry of Commerce, 2012).

Cultivation: Seeds of the palm are sown in fields. The palms grow and generally start to bear fruit at about the age of four to eight years. Flowering starts between November and February in India, or slightly earlier in Bangladesh. The flowers are mostly wind pollinated, and the fruits take about 8 months to fully ripen. Each bunch can yield anywhere between 50 and 400 fruits, and each palm can continue to produce fruits for 60 to 100 years.

Harvesting and processing: Betel vine is cultivated on a wooden frame to give it support. The seeds are harvested every year. Harvesters climb the palms that are in close and regularly spaced plantings. They cut the bunch lower them down on a rope and move from one crown to the other. Another method is to harvest bunches with a knife mounted on a bamboo pole. The stage of harvesting the seeds depends on the product wanted. Immature fruits supply 'kalipak', an important form of processed betel nut in India. These are picked when the fruits are 6-7 months old. For the mature nut product, fruits should be harvested fully ripe.

Table 2.1 *A. catechu* L. Cultivation Areas in Thailand

Rank	**District**	**Cultivation area (Rai)**	**Production (Kg/ Rai)**	**Total production (Kg)**
1	Chum-phorn	24,516	6,700.00	107,849.90
2	Nakorn Sri Thammarat	18,408	6,091.84	99,461.50
3	Ranong	13,556	3,430.74	31,230.04
4	Cha Chaerng Sao	9,583	1,958.40	17,641.30
5	Pattalung	6,451	6,700.00	34,786.40
6	Trang	4,649	6,700.00	28,387.90
7	Pang-nga	4,282	5,362.81	14,683.36
8	Rayong	4,048	2,089.88	7,026.19
9	Surat Thani	3,427	4,705.38	11,509.36
10	Nakorn Prathom	3,409	3,172.30	10,697.01

From Thai Department of Agriculture Extension. (2002). **อนาคตหมากไทยจะเป็นอย่างไร.** Retrieved August 10, 2011, from http://www.doae.go.th/library/ html/detail/

After harvesting, the fruits are husk removed, either while fresh or after drying, and the whole or sliced nuts are dried in the sun or with artificial heat. Ripe and almost ripe nuts are left whole or are sliced. They are sometimes boiled in water, which reduces the tanning content of the nuts, and then dried. The product is graded on the basis of the ripeness at harvesting and on the color, shape and size of the nuts. The most popular form is the dried, ripe, whole nut.

Food: The betel nut palm does not play much of a role in the food of South Asia. The seeds are sucked or chewed, but are not classed as a food. The seeds are added to some foods and sweets to provide an aromatic flavoring. In India and in Indian restaurants in Europe, betel nuts may be added to mukwas. These are mixtures containing aromatic seeds and fragments of sugary sweets. Dishes are often placed on Indian restaurant tables as an after-dinner nibble. Betel nuts may also be used to make paan masala in India. These are sort of breath-freshening ready-made mixes containing little pebbly little bits of betel nut dusted with powdered betel pepper (*Piper betel*) leaf and lime, and usually flavored strongly with menthol.

Ayurvedic medicine: Ayurvedic practitioners have a range of uses for betel nut. Ripe and unripe seeds are used to treat urinary infections and vaginal discharges. Ayurveda also recognizes betel nut as useful for relieving toothache, to aid digestion and as a laxative. It is reported to sweeten the breath, strengthen the gums and prevent perspiration. Some claim the plant has aphrodisiac properties too. The powdered seeds also feature in Unani medicine. They are prescribed for the treatment of diarrhea and urinary disorders.

Traditional and folk medicine: The seeds of the betel nut palm are best known for their use as a stimulant but traditional medicinal uses include relief from abdominal discomfort and eliminating intestinal parasites like roundworms and tapeworms. Betel nut's use in folk medicine extends to smallpox, cholera and sexually transmitted diseases such as syphilis. One traditional remedy claims that '*immortality can be attained by consuming a decoction of this plant*'. Externally the nuts are used for treating wounds, ulcers and sores.

Recent research: The main medicinal activities of the seed (also known as the nut) are reported to be anti-parasitic, laxative and to promote urination. It is also proven to be a stimulant since it increases both heart rate and blood pressure. The effects of betel nut on mental health are more controversial. By increasing glucose absorption in the brain, betel nut may be able to help those suffering from Alzheimer's disease. There are also hints that betel nut consumption might help sufferers from schizophrenia, but this is not yet demonstrated. Any medicinal use of betel nut will need to be balanced against its known harmful effects. The betel quid causes strong dental health, reduces teeth decay and discolors teeth and mouth, but also implicates as carcinogen since the polyphenols in areca seed can generate oxidative stress under alkaline condition (Huang, P.L., Chi, C.W. & Lui, T.Y., 2010). The tender shoot is also edible and cooked as syrup (Wang, C.W., Lee, W.H. & Peng, C.H., 1997). The seed is used as chewing material anthelmintic and also traditional medicine (Lee et al., 1999a,b) containing polyphenols; alkaloids (arecoline, arecaidine, guvacine, and guvacoline), tannins (phlobatannin and catechin), sugars and lipids (Wetwitayaklung et al., 2006) The pericarp also contains leukocyanidins, tannins, and lipids that exhibit antibacterial and antifungal properties (Yenjit, P., Issarakraisila, M., Intana, W. & Chantrapromma, W., 2010). Both leaves and seed were used in various preparation including treatment of diarrhea, dropsy, sunstroke, beri-beri, throat inflammations, oedema, lumbago, bronchia catarrh, and urinary disorders (Oxenham et al., 2002).

S*timulating action*: Betel nut is usually chewed with lime (calcium hydroxide) and betel pepper leaves. When mixed with the lime the arecoline in the betel nut is converted to arecaidine. This contributes to the various effects of betel quid on the central nervous system. Both arecoline and arecaidine have been shown to interfere with the action of some of the nerves in the brain, which results in the stimulant effects. The action of these alkaloids might also explain why betel nut affects the appetite. Compounds called tannins are also found in the fruit and it is these compounds that may help treat the symptoms of dysentery.

2.2 Biological Activities of *A. catechu* L.

There was the previous study of phenolics and alkaloid content in *A. catechu* Linn. with the various sample of fresh areca fruits of various maturities, upside-down areca fruit, tender shoot, root, leaf, spike, and vein of the tree (Wang et al., 1997). The phenolics in *A. catechu* Linn. samples were determined by Folin-Ciocaltue method and found mainly distributed in root followed by fresh unripe fruit, leaf, spike, and vein, respectively. The alkaloids content in *A. catechu* Linn. samples were determined by HPLC. The order was highest in root and lower in fresh unripe fruit, spike, leaf, and vein, respectively. Total amount of phenolics in areca fruit were well correlated with the length and maturation, but those alkaloids were only correlated with maturation. Upside-down areca fruit contained a much higher amount of arecaidine alkaloid (4 mg/g of fresh weight) than normal fresh unripe areca fruit (1.5 mg/g of fresh weight). There is no significantly different of phenolic group content (condensed tannins, hydrolyzable tannins, non-tannin flavans, and simple phenolic) between normal unripe fruit and upside-down fruit. Tender shoot contained a small amount of total phenolics (0.58 mg of gallic acid equivalent/g of fresh weight), condense tannin

(0.85 mg of catechin equivalent/g of fresh weight), and total alkaloids (2.38 mg/g of fresh weight).

Biological screening of 100 plant extracts for cosmetic use in the perspective of free radical scavenging activity (Kim B.J., Kim J.H., Kim, H.P. & Heo, M.Y., 1997) stated the methanolic extracts including *A. catechu* (seed and peel) were screened for anti-oxidative activity using Fenton's reagent/ethyl linoleate system (lipid peroxidation system) and for free radical scavenging activity using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical generating system. *A. catechu* peel showed no inhibition at a concentration of 10 µg/mL or 68% inhibition at 1,000 µg/mL in lipid peroxidation assay using the TBA method, and 9% inhibition at a concentration of 10 µg/mL or more than 24% inhibition at 1,000 µg/mL in DPPH free radical assay. The *A. catechu* seed showed 24% inhibition at a concentration of 10 µg/mL or 73% inhibition at 1,000 µg/mL in lipid peroxidation assay using the TBA method, and 52% inhibition at a concentration of 10 µg/mL or 47% inhibition at 1,000 µg/mL in DPPH free radical assay. The results suggest that *A. catechu* may be potential sources of anti-oxidants.

Free radical scavenging effect of ethanol extract from *A. catechu* L. was studied (Ahn, B.Y., 2009). The antioxidant activities of the ethanol extracts obtained from selected medicinal plants, which are frequently used as food and herb medicine, have been evaluated by measuring their DPPH radical scavenging activities. Among most plant extracts, the medicinal plant which showed the highest DPPH radical scavenging activity are the ethanol extract from semen of *A. catechu* L. follow by radix of *Usmus macrocarpa*, fryctus of *Terminalia chebula* Retz., Flos *santalum album* L., and semen of *Cassia tora* L. at IC_{50} of 4.5, 6.0, 7.0, 8.0, and 8.0 µg/mL, respectively. The ethanol extract of *A. catechu* was also shown to have stronger radical elimination ability than ascorbic acid and BHT.

The effects of *A. catechu* L. extract on anti-inflammation and anti-melanogenesis were studied (Lee & Choi, 1999a). The ethanolic extract from areca seed was prepared and its various biological activities were evaluated, the extract showed potent anti-oxidative (lipid peroxidation system), free radical scavenging (DPPH assay), and anti-hyaluronidase activity (anti-inflammatory inhibition of hyaluronidase). Anti-oxidative effect of the extract (IC_{50}, 45.4 µg/mL) was lower than butylated hydroxytoluene (IC_{50}, 5 μg/mL), but similar to tocopherol and higher than ascorbic acid, especially, the extract exhibited relatively high free radical scavenging activity (IC_{50}, 10.2 μg/mL) compared to control. The extract inhibited effectively hyaluronidase activity (IC_{50}, 416 μg/mL), showed inhibition *in vivo* on delayed hypersensitivity as well as croton-oil induced ear edema in mice when it was topically applied. These results strongly suggest that the extract may reduce immuno-regulatory/inflammatory skin trouble. Also, from the results, it can be elucidated that the extract showed anti-allergic and anti-cytotoxicity activity. The whitening effect of the extract was shown by the inhibition of mushroom tyrosinase activity with IC_{50} of 0.48 mg/mL and of melanin synthesis in B16 melanoma cells (Lee & Choi, 1999a). They also do the related research on the anti-aging effect of ethanolic extract from areca seed on skin both *in vitro* and *in vivo* (Lee & Choi, 1999b). The extract has a high proportion of proline (13%) of free amino acid content. The inhibitory effect on the elastase exhibited 37 to 90% inhibition by 10 to 250 μg/mL concentration; the IC_{50} values with 40.8 μg/mL for porcine pancreatic elastase (PPE) and 48.1 μg/mL for human leukocyte elastase, respectively. One of the effects of elastase is that it is known to reduce the number of elastin fibers at the level of enzyme deposition. The number of elastin fibers was increased when it was drifted from the deposit number of elastase by 100 μg/mL of the extract. The extract showed protection of elastin fiber against degradation by the enzyme in an *ex vivo* essay. The extract increased proliferation of human fibroblast cell by 85% at 10^{-4} concentration, compared with control, whereas the increase by ascorbic acid was 50%. The collagen synthesis was increased by 40% at 10.4% concentration of the extract. The treatment with the extract improved skin hydration, the skin elasticity and skin wrinkles. The results from (Lee & Choi, 1999b) were strongly suggested that the ethanolic extract from areca seed can be used as a new anti-aging component for cosmetics. Moreover, the anti-elastase and anti-hyaluronidase of phenolic substances from *A. catechu* L. as new anti-aging agent was reported (Lee, K.K., Cho, J.J., Park, E.J. & Cho, J.D., 2001). Purified ethanolic-aqueous extract from areca seed by each fraction of solvents (combination of solvent containing various proportions of ethanol, hexane, chloroform, ethyl acetate, and butanol), siliga gel column chromatography, preparative TLC, and reversed-phase HPLC were studied. The peak in HPLC, which coincided with the inhibitory activity against elastase, was identified as a phenolic substance by using various colorimetric methods, UV and IR. IC_{50} values of this phenolic substance were 26.9 µg/mL for porcine pancreatic elastase (PPE) and 60.8 µg/mL for human neutrophil elastase (HNE). This phenolic substance showed more potent activity than that of reference compounds, oleanolic acid (76.5 µg/mL for PPE and 219.2 µg/mL for HNE) and ursolic acid (31.0 µg/mL for PPE and 118.6 µg/mL for HNE). According to the Linewaver-Burk plots, the inhibition against both PPE and HNE by this phenolic substance was competitive inhibition with the substrate. The phenolic substance from *A. catechu* effectively inhibited hyaluronidase activity (IC_{50}, 210 µg/mL). These results suggest that the phenolic substance purified from *A. catechu* has an anti-aging effect by protecting connective tissue proteins (Lee et al., 2001).

The study of antioxidant capacity in various parts of *A. catechu* L. (Wetwitayaklung et al., 2006) found that the antioxidant activities in various ages of seed and various plant parts of areca related to their tannin percentage and total phenolic contents, and to find out which kind of solvent giving the highest antioxidant activities by TEAC method. Results showed that the water and methanol extracts of the seeds in various ages presented higher tannin percentage and phenolic content than the other parts of areca tree extract. The methanol extracts of the seeds in various ages gave higher antioxidant activities than the other parts (leaves, crown shafts, fruit shells; 4 and 8 months, root, and adventitious root) of areca tree extracts. The sequence of antioxidant activities of methanol seeds extracts from high to low were 4, 6, 8, 3, 2, and 1 month seed extracts. The 82.05% antioxidant activities assessed from phenolic compounds in areca, and arecoline indicated no antioxidant activity (Wetwitayaklung et al., 2006). *In vitro* antioxidant activity of ethanolic extracts of *Centella asiatica, Punica grenatum*, *Glycyrrhiza glabra*, and *Areca catechu* L. were reported (Ashawat, M.S., Shailendra, S. & Swarnlata, S., 2007). The effects of all ethanolic extracts of the herbs were studied via reducing power estimation method. The antioxidant activities of the extracts were compared with ascorbic acid standard and found that areca nut showed highest activity ($p<0.05$). These studies may suggest that the chemical combination with extract as antioxidant can be utilized in pharmaceutical and cosmetic formulation or chemical antioxidant replaced by herbal natural antioxidants (Ashawat et al, 2007).

The study of areca nut for its antimicrobial properties evaluated the antibacterial, antifungal, and antiviral properties of the areca nut in vitro using isolated organisms (Anthikat, R.R.N. & Michel, A., 2009). A variety of human veterinary isolates, both gram positive and gram negative, were tested against areca nut extract by measuring the growth of the organisms by spectrophotometric method. It was found that both gram negative and gram positive organisms were susceptible to the areca nut extract. The concentration needed for 100% inhibition of growth was found to be 3.3-7 µg/mL for gram negative bacteria and 16 µg/mL for gram positive bacteria. The extract was also found to inhibit the growth of *Candida albicans* at a concentration of 16 µg/mL and inhibited alfatoxin production by *Aspergillus flavus*. The extract was also found to inhibit the viral growth of the New Castle Disease Virus (NDV) and Egg Drop Syndrome Virus (EDS) grown in embryo cultures. These results indicate that betel nut chewing may have significant disinfective properties (Anthikat & Michel, 2009).

2.3 Free Radicals

Free radicals are known to be the major cause of various chronic and degenerative diseases, including aging, coronary heart disease, inflammation, stroke, diabetes mellitus and cancer (Cheng, H.Y., Lin, T.C., Yu, K.H., Yang, C.M. & Lin, C.C., 2003). Reactive oxygen species (ROS) include free radical such as O_2^- (superoxide anion), OH (hydroxyl radical, H_2O_2 (hydrogen peroxide) and $^1O_2$ (singlet oxygen) can cause cellular injuries and initiate peroxidation of polyunsaturated fatty acids in biological membranes. The tissue injury caused by ROS may include DNA damage, protein damage and oxidation of important enzymes in the human body. These events could consequently lead to the occurrence of various Free radical-related diseases. Any free radical involving oxygen can be referred to as reactive oxygen species (ROS). Oxygen centered free radicals contain two unpaired electrons in the outer shell. When free radicals steal an electron from a surrounding compound or molecule a new free radical is formed in its place. In turn the newly formed radical then looks to return to its ground state by stealing electrons with anti-parallel spins from cellular structures or molecules. Thus the chain reaction continues and can be thousand of events long. The electron transport chain (ETC), which is found in the inner mitochondrial membrane, utilizes oxygen to generate energy in the form of adenosine triphosphate (ATP). Oxygen acts as the terminal electron acceptor within the ETC. The literature suggests that anywhere from 2 to 5% of the total oxygen intake during both rest and exercise have the ability to form the highly damaging superoxide radical via electron escape. During exercise oxygen consumption increases 10 to 20 fold to 35-70 mL/ kg/ min. In turn, electron escape 4 from the ETC is further enhanced. Thus, when calculated, 6 to 3.5 mL/kg/min of the total oxygen intake during exercise have the ability to form free radicals (Halliwell, B., 2005).

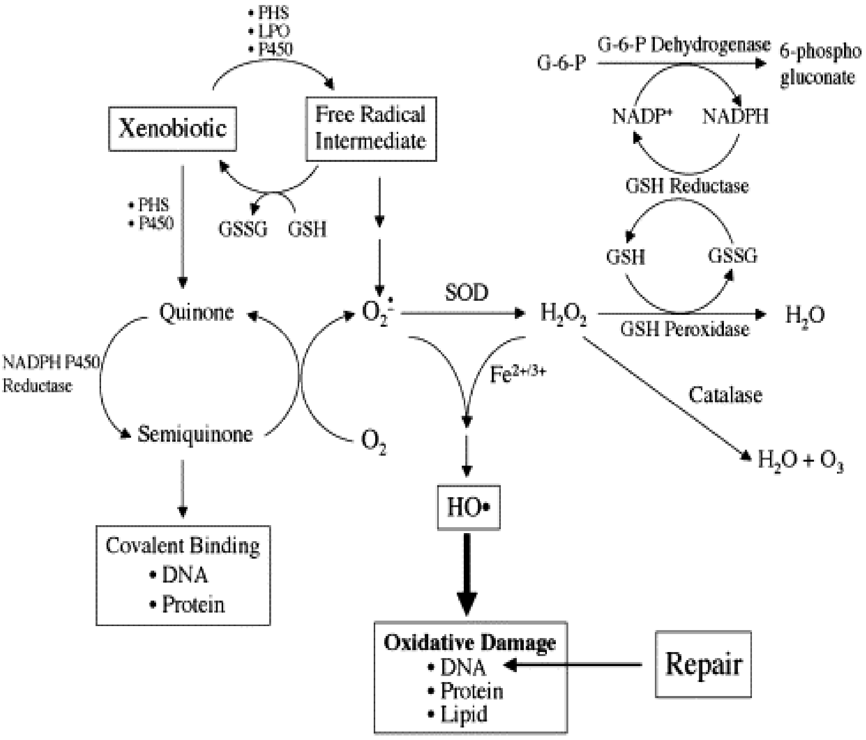

Figure 2.7 Pathways Contributing to the Formation and Detoxification of Reactive Oxygen Species (ROS)

There are numerous types of free radicals that can be formed within the body, especially, oxygen centered free radicals or ROS. The formulation of reactive oxygen species (ROS) is shown in Figure 2.7. In addition to the embryonic enzymatic bioactivation of xenobiotics to free radical intermediates, and the redox cycling of quinone metabolites, ROS also may be produced in the embryo (Wells, P.G., Bhuller, Y., Chen, C.S., Jeng, W., Kasapinavic, S., Kennedy, J.C., Kim, P.M., Laposa, R.R., McCallum, G.P., Nicol, C.J., Parman, T., Wiley, M.J. & Wong, A.W., 2005).

The most common ROS were shown in the equations below (Flora, S.J.S., 2009) including the hydroperoxyl radical ($HO_2^{\cdot}$), superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$).

$O_2+e^-+H^+$ ------------- $H0_2^{\cdot}$ (2.1)

$HO_2^{\cdot}$ ------------------- $H^+ +0_2\text{-}^{\cdot}$ (2.2)

$O_2\text{-}^{\cdot} + 2H^+ + e^-$ ------- H_2O_2 (2.3)

$H_2O_2 + e^-$ ------------- $OH^- + OH^{\cdot}$ (2.4)

$OH + e^- + H^+$ --------- H_2O (2.5)

The superoxide anions are formed when oxygen (O_2) acquires an additional electron, leaving the molecule with only one unpaired electron. They are derived from molecular oxygen under reducing conditions. However, because of their reactivity, these same free radicals can participate in unwanted side reactions resulting in cell damage. Excessive amounts of these free radicals can lead to cell injury and death, which results in many diseases such as cancer, stroke, myocardial infarction, diabetes and major disorders. Within the mitochondria O_2^- is continuously being formed. The rate of formation depends on the amount of oxygen flowing through the mitochondria at any given time.

$O_2^{-\bullet} \longrightarrow H_2O_2 \longrightarrow OH^{\bullet}$ (Harber Weiss Reaction)

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^-$ (Fenton Reaction)

Hydroxyl radicals are short-lived, but the most damaging radicals within the body. This type of free radical can be formed from O_2^- and H_2O_2 via the Harber-Weiss reaction. The interaction of copper or iron and H_2O_2 also produce $OH^·$ as first observed by Fenton. Singlet oxygen is not a free radical, but can be formed during radical reactions and also cause further reactions. When oxygen is energetically excited one of the electrons can jump to empty orbital creating unpaired electrons. Singlet oxygen can then transfer the energy to a new molecule and act as a catalyst for free radical formation. The molecule can also interact with other molecules leading to the formation of a new free radical.

2.4 Antioxidants

Antioxidant is increasingly popular in modern society as it gains publicity through mass media coverage of its health benefits. The dictionary definition of antioxidant is rather straightforward but with a traditional annotation (Huang, D., Ou, B. & Prior, R.L., 2005) a substance that opposes oxidation or inhibits reactions promoted by oxygen or peroxides, many of these substances (as the tocopherols) being used as preservatives in various products (as in fats, oils, food products, and soaps for retarding the development of rancidity, in gasoline and other petroleum products for retarding gum formation and other undesirable changes, and in rubber for retarding aging) (Huang et al., 2005). A more biologically relevant definition of antioxidants is synthetic or natural substances added to products to prevent or delay their deterioration by action of oxygen in air. In biochemistry and medicine, antioxidants are enzymes or other organic substances, such as vitamin E or β-carotene, that are capable of counteracting the damaging effects of oxidation in animal tissues (Huang et al., 2005). 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. Reactive oxygen species are produced through inflammation, the normal body process by which the body attacks foreign invaders and repairs itself. The body has a built-in ability to eventually neutralize these compounds, once they have done their work. When inflammation persists, the body’s own antioxidants may become depleted, allowing reactive oxygen species to accumulate and damage normal healthy cells. A common example of chronic inflammation is longstanding allergic dermatitis while a well-known and much feared consequence of reactive oxygen species is damage to DNA that results in the formation of cancer cells (Shalini, S., 2012). To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals.

There are 3 main types of antioxidant:

1) Nutrient-derived antioxidants: ascorbic acid (vitamin C), tocopherols (vitamin E), carotenoids, and other low molecular weight compounds such as glutathione, lipoic acid and other polyphenols compounds.

2) Antioxidant enzymes: superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions.

3) Metal binding proteins: ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions those are capable of catalyzing oxidative reactions.

Kinetically antioxidant is classifieds into 6 categories as below (Flora, 2009):

1) Antioxidants that break chains by reacting with peroxyl radicals having weak O-H or N-H bonds: phenol, napthol, hydroquinone, aromatic amines and aminophenols.

2) Antioxidants that break chains by reacting with alkyl radicals: quinones, nitrones, iminoquinones.

3) Hydroperoxide decomposing antioxidants: sulphide, phosphide, thiophosphate.

4) Metal deactivating antioxidants: diamines, hydroxyl acids and bifunctional compounds.

5) Cyclic chain termination by antioxidants: aromatic amines, nitroxyl radical, variable valence metal compounds.

6) Synergism of action of several antioxidants: phenol sulfide in which phenolic group reacts with peroxyl radical and sulphide group with hydro peroxide.

2.5 Phenolic Compounds

Phenolic compounds in plants are essential to the human diet, and are of considerable interest due to their antioxidant properties.

Table 2.2 Main Classes of Phenolic Compounds in Higher Plants

Classes and sub-classes	**Examples of specific compounds**
Non-flavonoids compounds	
Phenolic acids	
Benzoic acids	Gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid
Hydroxycinnamic acids	Coumaric acid, caffeic acid, ferulic acid
Hydrolyzable tannins	Pentagalloylglucose
Stilbenes	Resveratrol
Lignans	Secoisolaiciresinol, matairesinol, lariciresinol, pinoresinol
Flavonoid compounds	
Flavonols	Kaempferol, quercitin, myricetin
Flavones	Apigenin, luteolin
Flavanones	Naringenin, hesperetin
Flavanols	Catechins, gallocatechins
Anthocyanidins	Pelargonidin, cyaniding, malvidin
Condensed tannins or proanthocyanidins	Trimeric procyanidin, prodelphinidins
Isoflavones	Daidzein, genistein, glycitein

From Farah, A. & Donangelo, C. M. (2006). Phenolic compounds in coffee. **Brazil. J. Plant Physiol., 18**(1), 23-36.

These compound posses an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer. Flavonoids, which bear the C6-C3-C6 structure, account for more than half of the over eight thousand different phenolic compound. The antioxidant activity of phenolic compound depends on the structure, in particular the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings. Fruits, vegetables and beverages are the major sources of phenolic compound in the human diet. The food and agricultural products processing industries generate substantial quantities of phenolics-rich by-products, which could be valuable natural sources of antioxidants. Some of these by-products have been the subject of investigations and have proven to be effective sources of phenolic antioxidants. When tested in edible oils, and in fish, meat and poultry products, phenolic-rich extracts have shown antioxidant activities comparable to that of synthetic antioxidants. Practical aspects of extraction and production of sufficient amounts of natural antioxidants from most of these sources remain to be elucidated (Balasundram, N., Sundram, K. & Samman, S., 2006).

2.6 Phenolic Antioxidants Determinations

The multifaceted aspects of antioxidants and the basic kinetic models of inhibited autoxidation and analyzes the chemical principles of antioxidant capacity assays. Depending upon the reactions involved, these assays can roughly be classified into two types; assays based on hydrogen atom transfer (HAT) reactions and assays based on electron transfer (ET). The majority of HAT-based assays apply a competitive reaction scheme, in which antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds. These assays include inhibition of induced low-density lipoprotein autoxidation, oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), and crocin bleaching assays. ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes color when reduced. The degree of color change is correlated with the sample's antioxidant concentrations. ET-based assays include the total phenols assay by Folin−Ciocalteu reagent (FCR), Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP), total antioxidant potential assay using a Cu (II) complex as an oxidant, and DPPH. In addition, other assays intended to measure a sample's scavenging capacity of biologically relevant oxidants such as singlet oxygen, superoxide anion, peroxynitrite, and hydroxyl radical are also summarized. On the basis of this analysis, it is suggested that the total phenols assay by FCR be used to quantify an antioxidant's reducing capacity and the ORAC assay to quantify peroxyl radical scavenging capacity. To comprehensively study different aspects of antioxidants, validated and specific assays are needed in addition to these two commonly accepted assays (Huang et al., 2005).

2.6.1 Extractable Phenolic Content (EPC) Assay

The extractable content phenolic content of extracts was determined using to Folin-Ciocalteu method (Singelton, V.L., Orthofer, R. & Lamune-Raventos, R.M., 1999). The EPC were determined by the Folin-Ciocalteu reagent which contains molybdenum on superior oxidation state (Mo^{+6}) with a yellow color. Polyphenols compounds which contain at least one hydroxyl group (-OH) was determined its reducing activity of Mo^{6+} to inferior states of oxidation (Mo^{4+} or Mo^{5+}) which have a blue color and can be monitored spectrophotometry at 765 nm. Using gallic acid as standard total phenolic content and expressed as mg GAE/g dried weight sample.

2.6.2 Extractable Flavonoids Content (EFC) Assay

The EFC was determined by the theory of aluminium colorimetric assay. The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminium chloride forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids (Chang, C.C., Yang, M.H., Wem, H.M. & Chern, J.C., 2002). Using quercetin as standard total flavonoids content and expressed as mg QE/g dried weight sample.

2.6.3 DPPH Radical Scavenging Activity

Free radical scavenging is the generally accepted mechanism for how antioxidants inhibit lipid oxidation. The newly formed radical can mainly follow radical-radical interaction to render stable molecules, via radical disproportionate, although these secondary reactions are greatly hindered. It is important to notice that when studying plant extracts there is the existence of several possible radical scavenger species in the extracts. The capacity of biological reagents to scavenge the DPPH radical can be expressed as its magnitude of antioxidation ability. The DPPH alcohol solution is deep purple in color with an absorption peak at 517 nm, which disappears with the presence of the radical scavenger in the reactive system and when the odd electrons of the nitrogen in the DPPH are paired. The reactive rate and the ability of the radical scavenger depend on the rate and the peak value of disappearance of the DPPH (Li, Y.J., Chern, J., Li, Y., Li, Q., Zheng, Y.F., Fu, Y. & Li, P., 2011). The results of the DPPH assay have been presented in many ways. The majority of studies express the results as the IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%. This value is calculated by plotting inhibition percentage against extract concentration.

2.6.4 Ferric Reducing Antioxidant Power (FRAP)

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay (Benzie, I.F.F. & Strain, J.J., 1999). FRAP assay uses antioxidants as a reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. At low pH (3.6), the reduction of ferric-tripyridyl triazine (Fe^{3+}-TPTZ) complex has an intense color and can be monitored by measuring the change in absorption at 593 nm. The reaction is non specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous ($Fe^{3+}$to Fe^{2+}) ion formation. The change in absorbance is therefore, directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture (Benzie & Strain, 1996).

2.6.5 ABTS Radical Scavenging Activity

The 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) was pre-oxidized with potassium persulphate to generate $ABTS^{\bullet+}$. The mixture was incubated for 12-16 h to reach to complete reaction. The $ABTS^{\bullet+}$ stock was diluted with buffer for using in hydrophilic and with ethanol for lipophilic assay. The decolorizing reaction was monitored at 743 nm for radical scavenging activity of the antioxidant (Arnoa, M.B., Cano, A. & Acosta, M., 2001).

2.6.6 Lipid Peroxidation Inhibitory Activity

Lipid peroxidation is highly determined as its mechanism toxic to the oil, fatty acid and also lipid bilayer membrane, the basis structure of many cellular membranes. The presence of polyunsaturated fatty acids (PUFAs) in the lipid bilayer’s phospholipids critically impacted to the membrane fluidity. Lipid peroxidations affect to the membrane’s phase properties, electrical resistance, membrane protein moiety as well as lysosomes’s latency becoming fragile and leaky. Peroxidation also well-known to decrease membrane associated enzymes and membrane pump homeostasis maintenances. The major toxicological product been interested is 4-hydroxynonenal (4-HNE), various 2-alkenals, and malonaldehyde (MDA). In the perspective of food and dietary, rancidity of oil is the commonly lipid peroxidation event which is the oxygen-dependent deterioration of lipid. The major product LOOHs, the non radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, esters and cholesterol are formed from the reaction to the reactive oxygen species (ROS). The alkoxyl radical are formed as the result of the fission of cyclic endoperoxides (O-O bonds). The transition of metal complex catalyzes the forming of reduced iron compounds and react with LOOH, which is fairy stable molecules at physiological temperature. The interesting malonaldehyde (MDA) can be measured as an adduct formation with thiobarbituric acid (TBA). The resulted thiobarbituric reactive substance (TBARS) was spectrophotometrically monitored the pink color at 532 nm (Devasagayam, T.P.A., Boloor, K.K. & Rasmasarma., 2003).

2.6.7 Tyrosinase Inhibitory Activity

Recently the destruction of ozone layer leads to the more harmfulness of ultraviolet (UV) radiation. The excessive exposure to UV radiation causes post-inflammatory pigmentation. Pigmentary disorders are caused by many factors such as inflammatory, hormonal imbalance, and excessive exposure to UV (Lee & Choi, 1999a). The integrated mechanism for the formation and delivery of melanin within melanosomes from melanocyte to the keratinocytes (Moon, J.Y., Yim, E.Y., Sng, G., Lee, N.H. & Hyun, C.G., 2010) is to be considered. Melanin plays a critical role in UV absorption and free radical affects. The regular of cellular pigmentation can be controlled at many different stages of melanogenesis. The enzymatic producing process of melanin was involved with tyrosinase enzyme (Lee & Choi, 1999a). The process was depicted in Figure 2.8, Initial melanin synthesis is catalyzed by tyrosinase and is then divided into eumelanogenesis or pheomelanogenesis. The other melanogenic enzymes are involved in eumelanogenesis that is, l-3,4-dihydroxyphenylalanine (DOPA) chrome tautomerase (DCT) and tyrosinase-related protein 1 (TYRP1 and no specific enzymes have been found that are involved in pheomelanogenesis (Ando, H., Kondoh, H., Ichihashi, M. & Hearing, V.J., 2007). The spectrophotometry assays determined the inhibition of dopachrome melanin production via the inhibition of tyrosinase activity.

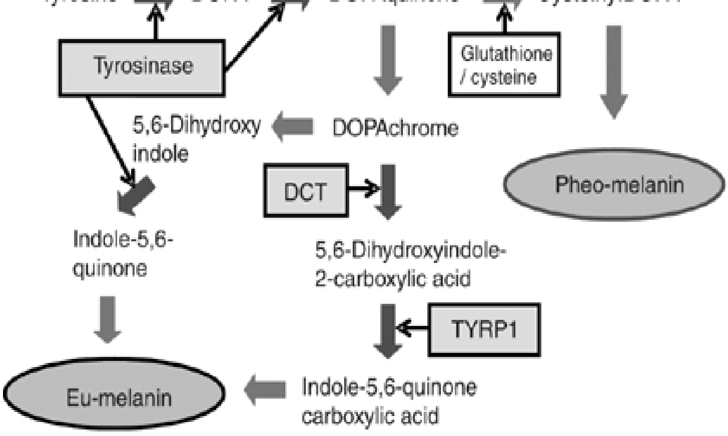

Figure 2.8 Melanin Synthetic Pathway and the Involvement of Melanogenic Enzymes

2.6.8 Elastase Inhibitory Activity

The skin elasticity significantly decreased as the age increased, especially significance at the age above 40 years old resulted in sagging. Biologically, elastase activity significantly increased with age (Lee & Choi, 1999a). Elastase is the only enzyme owning the elastin fiber breaking down property. The destruction of elastin leads to the destruction of skin demonstrates the skin-aging signs including sagging, wrinkles, and other fine lines (Moon et al., 2010). The elastase (EC 3.4.21.36) inhibition was assessing and N-succinyl-ala-ala-ala-*p*-nitroanilide was used as the substrate which specific to elastase. The hydrolysis by elastase prefers the alanine cleavage releasing the primary aromatic amine of *p*-nitroaniline. The colorimetric determination monitors the decrement of cleaved product by adding the inhibitor by the decrement of the absorbance at 510 nm.

2.6.9 Antibacterial Activity

The antibacterial activities of the plant extracts were widely study as the meaningful application as natural agent. The recent interesting strains to be inhibited are wide range depend on the application. The use in cosmetic product, antibacterial agents were purposed in both being the active ingredient and self preservative. The hygienic and sanitary cosmetics as well as anti-acne and cleansing products were inquired to be tested against the normal occurred strains. There were indicated bacteria which considered being avoided in cosmetic product. Since being the water containing formula, cosmetics contamination ability has been known. Microorganisms may cause spoilage or chemical changes in cosmetic products and injury to the user (Dashen, M.M., Chollom, P.F., Okechalu, J.N. & Ma-aji, J.A., 2011). The generally concerned bacterial are *Staphylococcus aureus*, *Staphylococcus epidermidis* (gram-positive bacteria) and *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. In addition to the mentioned bacteria, the occasionally occurrences of *Candida albican*, yeast and the recently regulation of *Clostridium* spp. contaminant in natural cosmetic products.

There were many testing method commonly employed in antimicrobial activity. The widely applied dilution method or agar disc and paper disc diffusion method were employed. The diffusion method was published being the most suitable for using in antimicrobial therapeutic test by the manufacturers. This well known method was nick named as the critical development by Bauer and Kirby (Bauer, A.W., Kirby, W.M.M., Sheris, J.C. & Turck, M., 1966). The inhibitory activity is observed by eyes and clear zone diameter measurement. The ability of the tested agents is compared to the synthetic antibiotic or drugs.

2.7 Extraction Method

The extraction process is one of the most concerned factors of developing natural extract. There are many factors including in the process of extraction. The target compound to be extracted and their nature and preferences was the firstly concern. The factor of extraction including, extraction method, extraction solvent, time, and temperature were in account of the extraction efficacy. The target compound has its own preferences. The volatile oil may extracted more in the high temperature accompany with the closed extraction apparatus like soxhlet or distillation clevenger. While the thermolabile substance like secondary metabolites are considered to extract with the less thermal involved method like conventional shaking or ultrasonication at room temperature to avoid the decomposition of the target compounds.

The extraction solvents are generally chosen by the solubility properties and the polarity index (Table 2.3) matching to the target compounds. The low polarity oil or fatty acid may be chosen to be extracted with low polar organic solvent such as hexane and petroleum ether while the medium polarity compounds appreciate dichloromethane chloroform or ethyl acetate. The higher polarity compound of polyphenols will be extracted more in higher polar organic solvent of acetone, ethanol, methanol or water (Kumoro, A.C., Hasan, M. & Singh, H., 2009). Many studies also literate the synergistic effect of the combination of two or more solvent in the system.

The alternative extraction method microwave assistance (MAE) was employed in the recent study. MAE was currently regarded as a robust alternative to traditional extraction techniques, especially in the case of the sample preparation for analytical purpose (Zhang, H.F., Yang, X.H. & Wang, Y., 2011). The advantages of MAE are, for example, suitable for the recovery of vast array of compounds, versatile and efficient for secondary metabolites extraction. Compared to the classical reflux and soxhlet extraction MAE shows the shorter extraction time, higher extraction yield, selectivity, and better quality target compounds (Mandal, V., Mohan, Y. & Hemalatha, S., 2007: Chen, Y., Xie, M.Y. & Gong, X.F., 2007). The cost-effective is one of the most factor concerning in the extraction development. MAE possessed lower cost compared to the supercritical fluid extraction, lower solvent consumption than accelerated solvent extraction and lower extraction time than ultrasonic-assisted extraction and suit to the thermolabile constituents (Zhang et al., 2011; Mandal et al., 2007). However, there were the demerit reports of MAE with the cooperated with high pressure (Zhang et al., 2011). Flavonoids extraction was found having higher degradation rate of rutin, hyperin, quercetin, and quercitin when the pressure in the system increased (Liu, Z.Y., Hu, X.L., Bu, F.Q., Ding, L. & Zhang, H.Q., 2007). Moreover, extraction efficacy of MAE may be poor when either extraction solvent or target compounds are non-polar, or when the viscosity of solvent is extremely high (Wang, L. & Weller, C.L., 2006).

Numbers of literatures, more than 20 reports stated the application of MAE combined with various additional functions such as focused oven, closed vessel, ultrasound, dry-diffusion system, and the simple domestic microwave oven as well. The potential extraction efficacy of microwave is the disruption of plant tissues and/or cells (even organelles) (Zhang et al., 2011). The plant material particle size impacted to the extraction efficacy as the smaller size allow more specific surface area of the sample to be extracted by solvent via microwave assistance (Gao, M., Huang, W., Moytri, R.C. & Lui, C., 2007). The same research also reported the different structure of plant material extracted by MAE and heat-reflux extraction, MAE possessed higher cell disruption observed by light micrographs (Gao et al., 2007).

Table 2.3 Some of the Solvent Polarity Index and Dielectric Constant

Solvent	**Polarity index**	**Dielectric constant**
Hexane	0.1	1.89
Ethyl acetate	4.4	6.08
Isopropanol	3.9	19.9
Acetone	5.1	20.7
Methanol	5.1	32.6
Ethanol	5.2	24.3
Acetronitrile	5.8	37.5
Water	9.0	78.3

Moreover, the extraction solvent employed in MAE is to be considered, in addition to the polarity, of its dissipation factor or the ability of the solvent to absorb microwave energy and pass it on as heat to the surrounding molecule (Mandal et al., 2007). The factors in calculated, dielectric loss and dielectric constant (Table 2.3) are also important to the extraction manner of the solvent. The former indicates the efficacy of converting microwave energy into heat and, more in considered in this study, the later is the ability of the solvent to absorb microwave energy.

2.8 Cosmetic and Multifunctional Agent

The world wide cosmetic market has been raised gradually. Thai Ministry of Commerce recently reported the 2012 cosmetics export value of Thailand was 82,747.2 million baht which expanded about 10% from 2011 (Thai Ministry of Commerce, 2012). Thai cosmetic product market segmentation of the skin care was of 41% for facial product. The lightening product was 48%, 43% for anti-aging and moisturizing, and another 10% for sun-protection and others (Marketing Oops, 2008). Skin care product in Asian countries also has a decidedly more dominant market share of 40% in both Japan and China market (Global Insight, 2007). Moreover, the consumer demands interested in the development of the product contains natural ingredients and the environmental friendly products. The Green Marketing in Europe, America, and Asia was the main export market of Thailand. Thai cosmetic product, approximately 70%, was recognized to be natural products. The worldwide natural product was investigated having the market value over 3 trillion baht (Kasikorn Research, 2010).

The main market share was of skin care product. The major attractiveness of consuming Thai cosmetic product was the lower cost with the high unique concept. The important export market was Asian countries of more than 40% of total share because of the similar perspective of natural and organic products. The second market share was of Japan (24.85%) who also interesting in natural concept. European countries (5.68%) and U.S.A. (0.68%) also interested in Thai cosmetic products (Thai Ministry of Commerce, 2012). The major expectation of the consumer from Thai cosmetic is the natural and safety cosmetic. The export value of natural extracts in 2012 was also reported by Thai Ministry of Commerce of 164.2 million baht raised more than 18% from 2011. Asian countries still being the main market of 58.52% and the lower was U.S.A. (14.24%). Europe and Japan also being where Thai natural extracts taken the export value (3.05% and 3.82%, respectively) (Thai Ministry of Commerce, 2012). The raising in export value indicated the gradually interesting in natural perspective. Cosmetics were one of the main products developed to be the natural concept. Including the natural active ingredients is one of the best claims. Thailand native plants and herbals were widely researched and developed. Natural extracts widely stated of their various cosmetic activity including, antioxidant, anti-melanogenesis, antibacterial, and moisturizing effects. The safety in use of natural was competitive with the synthetic one.

In addition, the cosmetic import value also critically raised to over 25% which will be considerable of wasting more than 20,927 million baht to the Europe (34.08%), U.S.A. (18.39%), Asian (14.07%), and Japan (13.18%) (Thai Ministry of Commerce, 2012). The Thai cosmetic research and development with adding the unique Thai concept serves the sustainable agriculture as well as the consuming of Thai cosmetic.

The multifunctional cosmetic also widely claimed as the simultaneous effects which suitable to the recent life style of the consumers. The effects can be, for example, moisturizing, anti-acne, anti-wrinkle, whitening, anti-bacteria, sunscreen, and also as the colorant properties. These can be obtained from the ingredients both as the basic, active or additive ingredients in the formulation. These inquiries lead the research and development of the natural multi-functional agent which achieve more than those synthetic chemical agents in the safety perspective.