CHAPTER 2

Literature review

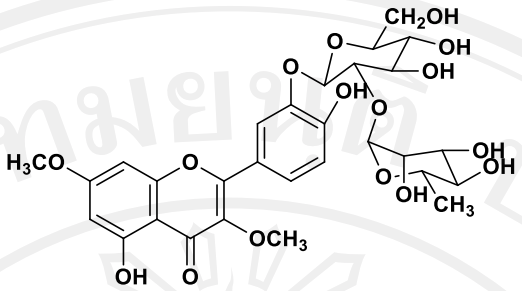
2.1 *Dasymaschalon*[12]

The genus *Dasymaschalon* is a small genus in the family Annonaceae. It is known in Thailand as "Bu-rong". This genus has about 40 species distributed in Southeast Asia, particularly in Thailand and Malaysia Peninsular. Twelve species were reported in Thailand that were *D. acuminatum, D. angustifolium, D. dasymaschalum, D.echinatum, D. filipes, D. glaucum, D. grandiflorum, D. lomentaceum, D. macrocalyx, D. obtusipetalum, D. sootepense,* and *D. wallichii*.

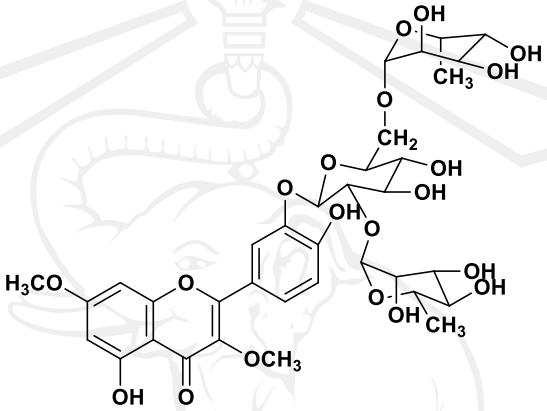
2.2 Chemical constituents of genus *Dasymaschalon*

The literature reviews focusing on the isolation of genus *Dasymaschalon* are shown below;

In 1998, Sinz and co-worker[10] isolated quercetin 3,7-dimethyl ether 3'-0-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (1) and quercetin 3,7-dimethyl ether 3'-0-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (2) from methanol extract of the leaves of *D. sootepense* by column chromatography and preparative high performance chromatography (HPLC).



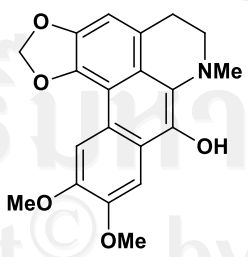
quercetin 3,7-dimethyl ether 3'-0-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (1)



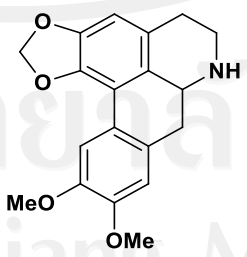
quercetin 3,7-dimethyl ether 3'-0-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (2)

Figure 2.1 Flavonol glycosides from *D.sootepense*

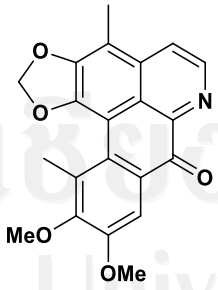
In 2008, six alkaloids; 7-hydroxydehydrodicentrine (3), nordicentrine (4), dicentrinone (5), sinactine (6), epiberberine (7) and aristolactam AII (8) were isolated by Hongtong and co-workers[3] from the ethyl acetate fraction partitioned from the methanol extract of the leaves and twigs of *D.sootepense*. Their structures were identified by spectroscopic techniques.



7-hydroxydehydrodicentrine (3)

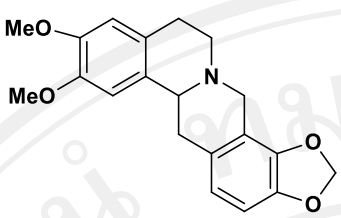


nordicentrine (4)

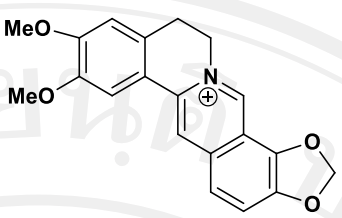


dicentrinone (5)

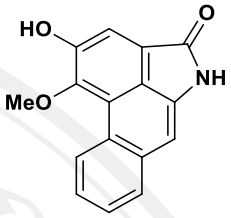
Figure 2.2 Aristolactam alkaloids from *D. sootepense*



sinactine (6)



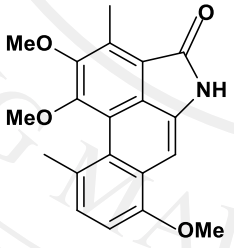
epiberberine (7)



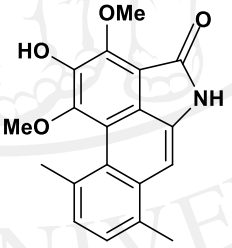
aristolactam AII (8)

Figure 2.3 Aristolactam alkaloids from *D. sootepense* (continued)

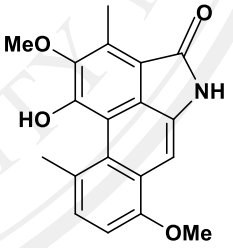
In 2011, Chanakul and co-workers[4] isolated cytotoxic alkaloids from the ethyl acetate extract of stems, leaves and twigs of *D. blumei*. Stems were separated by column chromatography and identified by spectroscopic techniques. Aristolactam alkaloids; aristolactam BI (9), goniopedaline (10), griffithinam (11) and 3,5-dihydroxy-2,4-dimethoxyaristolactam (12) were found. Moreover, oxoaporphine alkaloids; dicentrinone (5), oxodiscoguattine (13) and duguevalline (14) were isolated from leaves and twigs. Three aristolactam alkaloids; (9), (10) and (12) are cytotoxic against mammalian cancer cell lines and did not effect the viability of the noncancancerous human embryonic kidney cell (Hek 293).



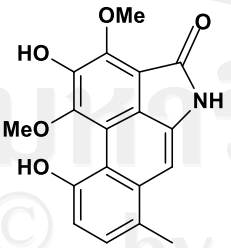
aristolactam BI (9)



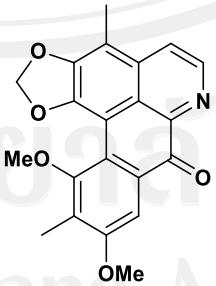
goniopedaline (10)



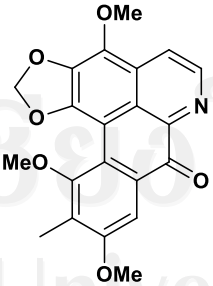
griffithinam (11)



3,5-dihydroxy-2,4-dimethoxyaristolactam (12)



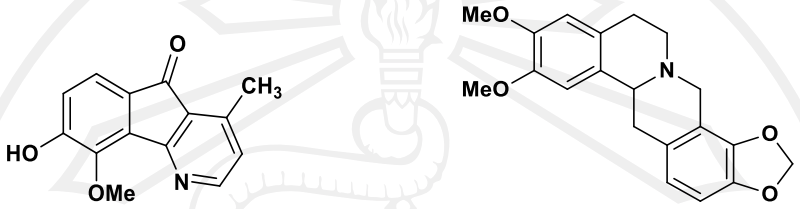
oxodiscoguattine (13)



duguevalline (14)

Figure 2.4 Aristolactam and oxoaporphine alkaloids from *D. blumei*

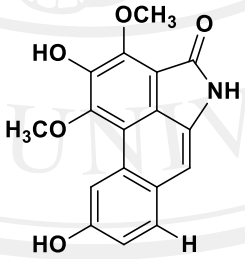
In 2011, four alkaloids; dicentrinone (5), epiberberine (7), isoursuline (15) and tetrahydroepiberberine (16) were found in the methanol extract from leaves and twigs of *D. glaucum* by Karntanakrit and co-workers[5]. The structures of all isolated alkaloids were determined by means of the spectroscopic methods and were confirmed with the data previously reported in the literatures.



isoursuline (15) tetrahydroepiberberine (16)

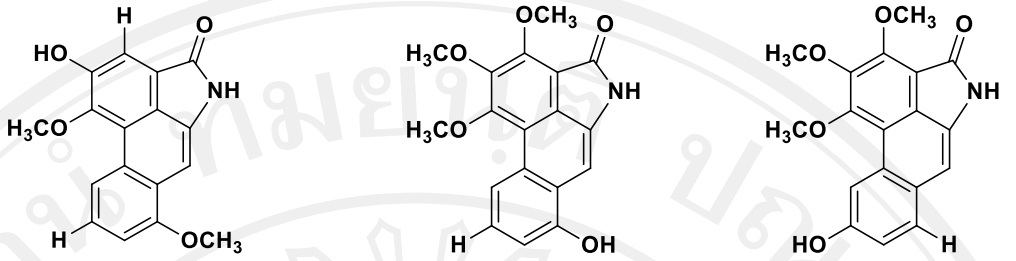
Figure 2.5 Alkaloids from *D. glaucum*

In 2013, Xiao-Lei and co-workers[6] isolated a new aristolactam alkaloid which is 10-Amino-3,6-hydroxy-2,4-dimethoxyphenanthrene-1-carboxylic (17) acid lactam and four known aristolactam alkaloids; enterocarpam-II (18), oldhamactam (19), goniopedaline (10) and stigmalactam (20) from stems of *D. trichophorum*.



10-Amino-3,6-hydroxy-2,4-dimethoxyphenanthrene-1-carboxylic (17)

Figure 2.6 Aristolactam alkaloids from *D. trichophorum*



enterocarpam-II (18) oldhamactam (19) stigmalactam (20)

Figure 2.7 Aristolactam alkaloids from *D. trichophorum* (continued)

2.3 *D. obtusipetalum*[13]

The previous reports indicated that alkaloid is the major compound of this genus. In this research, alkaloids from *D. obtusipetalum* will be focused.

Morphology : small tree, 12 m tall, 5 cm d.b.h. Young branches sparsely hairy. Leaf laminas 12.5-17 cm long, 3.5-5.5 cm wide, length/width ratio 2.8-3.5, elliptic to oblong, apex acuminate, base slightly cordate, chartaceous, glabrous ad- and abaxilly, glaucous abaxially; midrib glabrous and slightly impressed adaxially, sparsely hairy abaxially; secondary veins 8-14 per side of leaf, flush or impressed adaxially; petioles 5-7.5 mm long, 1.4-1.8 mm in diameter, sparsely hairy. Flowers on young growth; flowering pedicels 46-48 mm long, 0.9-1.2 mm in diameter, sparsely hairy. Sepals 2-4 mm long, 2.5-3 mm wide, length/width ratio 0.9-1.8, triangular, 0.3-0.4 mm thick, glabrous adaxially, hairy abaxially. Stamens ca. 130 per flower, 1.7-2.6 mm long; connectives truncate, ca. 0.5 mm long, 0.7-0.9 mm wide, glabrous; pollen exine echinate. Fruiting pedicels 22-63 mm long, 1.5-2.7 mm wide, sparsely hairy. Monocarps 9-14 per fruit, 25-65 mm long, 6.5-7.5 mm wide, ellipsoid (1-seeded) or moniliform (> 2-seeded), sparsely hairy, red; constriction 2-2.5 mm wide (25-35% of monocarp width); apicule 1.2-3.5(-7) mm long; stripes 6.5-9.5 mm long, 1.5-2.3 mm in diameter, sparsely hairy. Seeds 1-4 per monocarp, 13-16 mm long, 6-7 mm wide, length/width rayio 1.8-2.5, ellipsoid, brownish-yellow; hilum circular or elliptic, 1.8-2 mm indiameter; raphe prominent. Morphology of *D.obtusipetalum* as shown in Figure 2.8.

Distribution and Habitat : Northen Thailand (Doi Tung, Chiang Rai Province and Doi Sutep, Chiang Mai province). Mountane forest over limestone; 800-1600 m.

Phenolohy : Flowering specimens collected in May to June. Fruiting specimens collected in February, March, May and November

Thai Vernacular Name : Bu–rong Dok Thu

Figure2.8 Leaves and flower of *D. obtusipetalum*

2.4 Alkaloid Extractions[14]

A general process of alkaloid extraction using acid base extraction. The alkaloids are organic bases similar to the alkalies (inorganic bases); the name means alkali-like. The plant material often contains substantial quantities of fats (this is particularly true for the seeds), and also waxes, terpenes, pigments, and other lipophilic substances, which may interfere with the extraction procedure, for example, by causing the formation of emulsions. These technical problems can be more or less completely avoided by a preliminary defatting of the crushed drug. Petroleum ether and hexane are well suited for this step: alkaloids are soluble in these solvents only in exceptional cases, when the medium is neutral.

The powdered defatted drug is mixed with an alkaline aqueous solution that displaces the alkaloids from their combinations as salts; the free bases are then extracted with an organic solvent. Alkalinization is very often achieved with aqueous ammonia. If the structure of the alkaloids to be extracted contains a fragile element, for example, an ester or lactone function, aqueous ammonia must be replaced by an alkaline carbonate

solution. The organic solvent can be a chlorinated solvent (dichloromethane, chloroform), ethyl acetate, or diethyl ether.

The organic solvent containing the alkaloids as bases is separated from the residue and if necessary, partially concentrated by distillation under reduced pressure. The solvent is then stirred with an acidic aqueous solution, the alkaloids go into solution in the aqueous phase as salts, whereas the neutral impurities remain in the organic phase. The operation is repeated as many times as necessary until the organic phase no longer contains any alkaloids. Many acids are used (e.g., hydrochloric, sulphuric, sulphamic, citric, tartaric), but always in very dilute solutions (1-5%).

The aqueous solutions of the alkaloid salts, combined, and if necessary, "washed" with an apolar solvent (hexane, diethyl ether) are alkalinized with a base in the presence of an organic solvent immiscible with water. The alkaloids as bases precipitate and dissolve in the organic phase. The extraction of the aqueous phase continues until the totality of the alkaloids has gone into the organic phase. Finally, the organic solvent containing the alkaloids as bases is decanted, freed from possible traces of water by drying over an anhydrous salt (for example, sodium sulphate), and evaporated under reduced pressure. A dry residue is left: the total basic alkaloids.

2.5 Electrocoagulation (EC)

EC is an application of electrolysis technique which is useful for clarifying and decolourising certain solutions containing unwanted dissolved substances or suspended matter. It can remove a wide variety of pollutans, such as heavy metals[15, 16], various anions[17], oil-shale[18] and pesticides[19]. However, those solutions are mostly found in natural water, for example, food wastewater[20], dye-containing textile wastewater[21] and tennery wastewater[22]. Moreover, EC is currently applied to isolation many compounds from natural sources such as alkaloids[23-25], phenolic compounds[26], triterpenoids[27], polyhydroxy compounds[28] and glycosides[29] because this technique consumed less chemicals and organic solvents, especially those that are harmful to

environment than conventional technique. Conventional technique use large amounts of various organic solvents. These organic solvents are usually not only costly and toxic, but also eventually a burden to the environment.

Coagulation is a phenomenon which the charged particles in colloidal suspension are neutralized by mutual collision with counter ions and agglomerated, followed by sedimention. Coagulation is brought about primarily by reduction of the net surface charge to a point where the colloidal particles. Previously, stabilized by electrostatic repulsion, can approach closely enough for van der Waal forces to hold them together and allow aggregation. The reduction of the surface charge is a consequence of the decrease of repulsive potential of electrical double layer by the presence of electrolyte having opposite charge[30]. In EC process, the coagulation is generated by electrolytic oxidation of an appropriate anode material. Charged ionic species of metals or otherwise are removed from wastewater by allowing them to react with ions having opposite charge or the floc of metallic hydroxides generated within the effluent.

2.5.1 Theory of EC[30]

The theory of EC is generally accepted that the EC process involves three successive stage: (a) formation of coagulants by electrolytic oxidation of the 'sacrificial electrode'; (b) destabilization of the contaminants, particulate suspension and breaking of emulsions; and (c) aggregation of the destabilized phases to form flocs. The destabilization mechanism of emulsion may be summarized as follows:

2.5.1.1 Compression of the diffuse double-layer around the charge speciesvwhich is achieved by the interactions of ions that generated by dissolution of the sacrificial electrode due to passage of current through the solution.

2.5.1.2 Charge neutralization of the ionic species present in wastewater which is caused by the counter ions that produced by the electrochemical dissolution of the sacrificial electrode. These counter ions reduce the electrostatic interparticle repulsion sufficiently which the van der Waals attraction predominates that causing coagulation. A zero net charge results in the process.

2.5.1.3 Floc formation of coagulation creates a sludge blanket the entraps and bridges colloidal particles that have not been complexed.

2.5.2 Reaction types in the EC process[30-32]

The reaction of EC is highly dependent on the chemistry of the aqueous medium, especially conductivity. In addition, orter characteristics such as pH, particle size and chemical constituent will also influence to EC. Thus, reaction types of EC will be explained with two specific examples involving aluminum and iron which two metals have been extensively used.

1) Aluminun (Al)

For aluminum (Al) as electrodes; at the anode, the oxidation of aluminum occurs causing the electrolytic dissolution of the aluminum anode which produces the cationic monomeric species such as Al^{3+} and $Al(OH)^{+2}$. At appropriate pH values they are transformed initially into $Al(OH)_3$ and finally polymerized to $Al_n(OH)_{3n}$ according to the following reactions:

$$Al \rightarrow Al^{3+} + 3e^-$$

$$Al^{3+} + 3H_2O \rightarrow Al(OH)_3 + 3H^+$$

$$nAl(OH)_3 \rightarrow Al_n(OH)_{3n}$$

However, depending on the pH of the aqueous medium, other ionic species, such as $Al(OH)^{2+}$, $Al_2(OH)_4^{2+}$ and $Al(OH)^{4-}$ may also be present in the system. Examination of the pE-pH equilibrium diagram reveals that , under appropriate conditions, various forms of charged multimeric hydroxo Al^{3+} species may be formed. For example, the structures of dimeric and polymeric Al^{3+} hydroxo complexes are shown below:

(A) (B)

Figure 2.9 The structure of dimeric (A) and polymeric Al^{3+} hydroxo complexes (B)[30]

These gelatinous charged hydroxo cationic complexes can effectively remove pollutants by adsorption to produce charge neutralization and enmeshment in a precipitate.

At the cathode, the reduction of water generally occurs as usual and the main reaction encountered in cathodic compartment is as follows:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$$

Other reaction types may be observed at hight pH (pH>8) near the cathode and provoke the precipitation of the carbonate salt on this electrode.

$$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O$$

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3$$

$$2CO_3^{2-} + Na^+ \rightarrow Na_2CO_3$$

Thus, the summaries of mechanism have been proposed for the production of $Al(OH)_3$ below:

Anode : $Al_{(s)} \rightarrow Al^{3+}_{(aq)} + 3e^-$

Cathode : $2H_2O_{(l)} + 2e^- \rightarrow H_{2(g)} + 2OH^-_{(aq)}$

Overrall : $2Al_{(s)} + 6H_2O_{(l)} \rightarrow 2Al(OH)_{3(s)} + 3H_{2(g)}$

2) Iron (Fe)

For iron (Fe) as electrodes; iron upon oxidation in electrolytic system produces iron hydroxide, Fe(OH)n, where n = 2 or 3. Two mechanisms have been proposed for the production of Fe(OH)n below:

Mechanism 1 (n = 2)

Anode : $Fe_{(s)} \rightarrow Fe^{2+}_{(aq)} + 2e^-$

$Fe^{2+}_{(aq)} + 2OH^-_{(aq)} \rightarrow Fe(OH)_{2(s)}$

Cathode : $2H_2O_{(l)} + 2e^- \rightarrow H_{2(g)} + 2OH^-_{(aq)}$

Overrall : $Fe_{(s)} + 2H_2O_{(l)} \rightarrow Fe(OH)_{2(s)} + H_{2(g)}$

Mechanism 2 (n = 3)

Anode : $4Fe_{(s)} \rightarrow 4Fe^{2+}_{(aq)} + 8e^-$

$4Fe^{2+}_{(aq)} + 10H_2O_{(l)} + O_{2(g)} \rightarrow 4Fe(OH)_{3(s)} + 8H^+_{(aq)}$

Cathode : $8H^+_{(aq)} + 8e^- \rightarrow 4H_{2(g)}$

Overrall : $4Fe_{(s)} + 10H_2O_{(l)} + O_{2(g)} \rightarrow 4Fe(OH)_{3(s)} + 4H_{2(g)}$

The Fe(OH)n(s) formed remains in the aqueous steam as a gelatinous suspension, which can remove the pollutants from wastewater by complexation followed by coagulation. In the surface complexation, the pollutant acts as a ligand (L)

to chemically bind hydrous ion and the prehydrolysis of Fe^{3+} cations lead to the formation of reactive clusters for treatment. The reaction are shown below:

$$L - H_{(aq)}(OH)OFe_{(s)} \rightarrow L - OFe_{(s)} + H_2O_{(l)}$$

2.5.3 Instumental set-ups for EC[30,31]

An electrocoagulating reactor may be made up an electrolytic cell with a pair of electrodes with one anode and one cathode, which are connected to a DC supplier (an external power source), as shown in Figure 2.10**.**

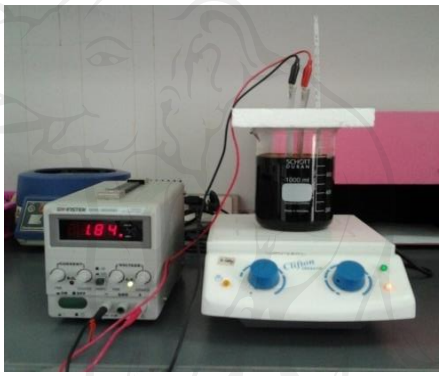


Figure 2.10 A simple electrocoagulation set-ups

These electrodes are usually metal plates, the positive one (the anode) being commonly known as the "sacrificial electrode". A small amount of supportive electrolyte in the form of salt (NaCl) is normally added to the aqueous medium for efficient flow of the current. When electricity is then passed into the electrolysed liquid via two electrodes. The anode will electrochemically carrode due to oxidation and the cathode will be more or less uncharged[29]. For larger electrolyzing containers, the used of electrodes with large surface area is required. This has been achieved by using cells with monopolar electrodes either in parallel or series connections. A simple arrangement of an EC cell with a pair of anodes and a pair of cathodes in parallel arrangement is shown in Figure 2.11.

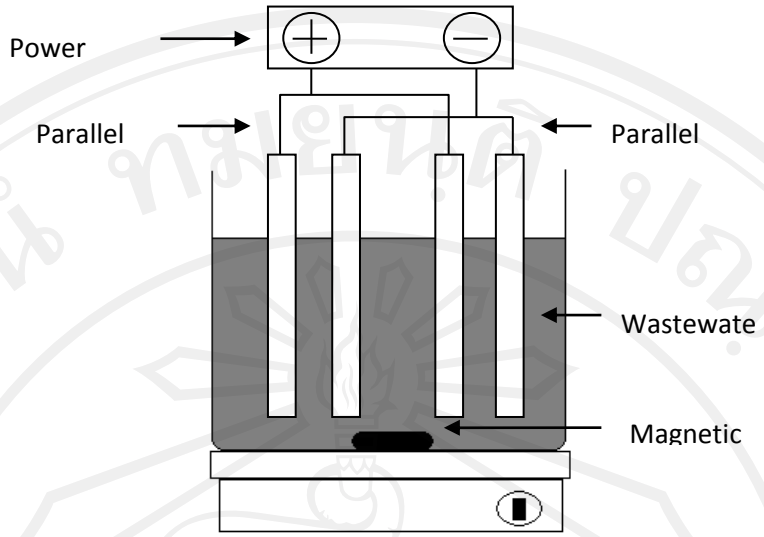


Figure 2.11 Bench-scale EC reactor with monopolar electrodes in parallel connection[30]

An arrangement of EC cell with monopolar electrodes in series, as shown in Figure.2.12. Each pair of the inner electrodes is internally connected with each other and has no interconnections with the outer electrodes. This arrangement, a higher potential difference is required for given current because the cells connected in series have higher resistance.

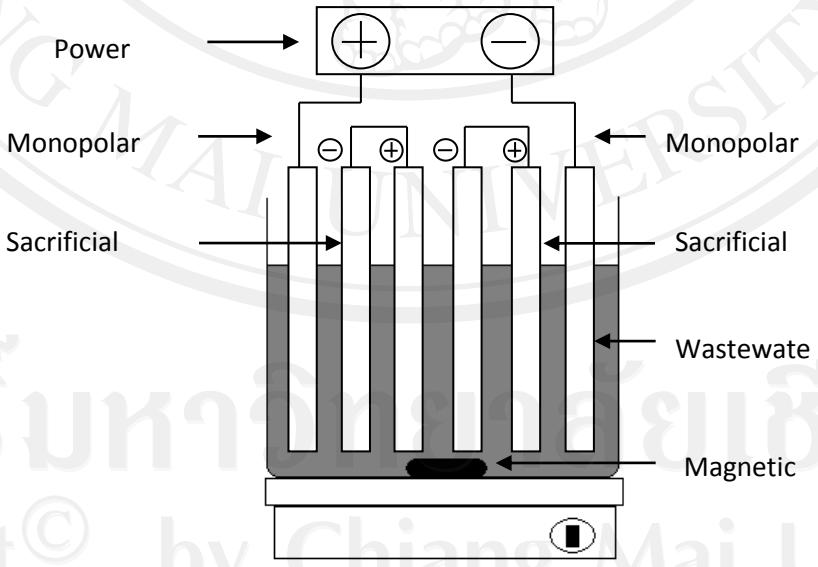


Figure 2.12 Bench-scale EC reactor with monopolar electrodes in series connection[30]

Another arrangement used bipolar electrodes, as shown in Fig. 2.13. The inner metal plates as bipolar electrodes without electrical connection with each orter. This cell provides a simple set-up which facilities easy maintenance during use. When an electric current is passed through the two electrodes, the neutral side of the conductive plate will be transformed to charged, which have opposite charge compared to the parallel side beside it.

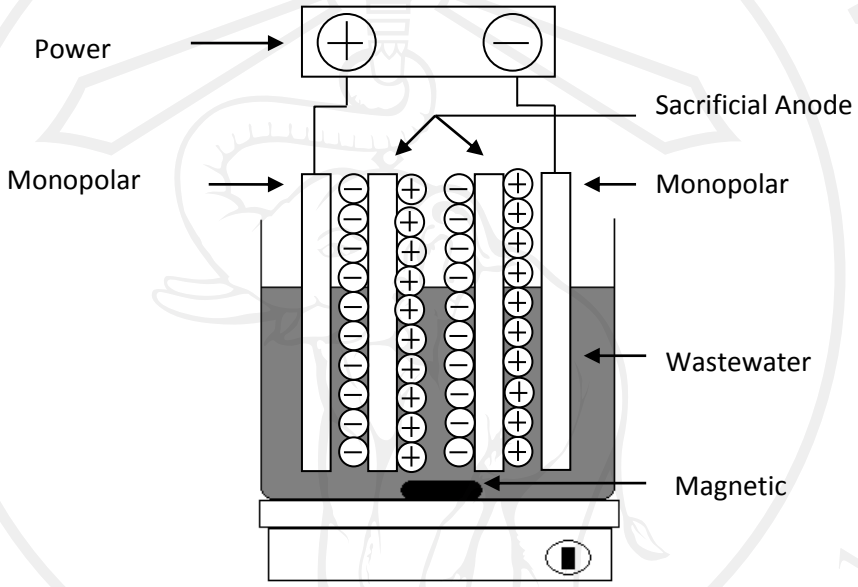


Figure 2.13 Bench-scale EC reactor with bipolar electrodes[30]

2.5.4 Advantages and Disadvantages of EC[30,31]

2.5.4.1 Advantages of EC

1. EC requires simple equipment and easy to operate with sufficient operational latitude to handle most problems encountered on running.

2. EC is at least as efficient as chemical coagulation with respect to removal of unwanted particles from the aqueous medium. In many case, EC is more efficient than chemical coagulation.

3. EC can remove the smallest colloidal particles because the applied electric field sets them in faster motion, thereby facilitating the coagulation. Wastewater treated by EC gives palatable, clear, colourless and odorless water.

4. Sludge formed by EC, being composed of mainly metallic oxides/ hydroxides, tends to be readily settable and easy to de-water.

5. The gas bubbles produced during the EC process can carry some of the pollutants to the top of the solution where it can be more easily concentrated, collected and removed.

6. The electrolytic processes in the EC cell are controlled electrically with no moving parts, thus requiring less maintenance.

7. No excessive chemicals are used or produced in the EC process and so there is no problem of neutralizing excess chemicals and no possibility of secondary pollution caused by chemical substances added at high concentraton as when chemical coagulation of wastewater is used. Thus, EC produces effluent with less total dissolved solids (TDS) content as compared with chemical treatments.

8. Flocs formed formed by EC are similar to chemical floc but EC floc tends to be larger, contains less bound water, is acid-resistant and more stable.Thus it can be separated faster by filtration.

9. The EC process avoids uses of chemicals, there is no problem of neutralizing excess chemicals and no possibility of secondary pollution caused by chemical substances added at high concentration as when chemical coagulation of wastewater is used.

10. The EC technique can be conveniently used in rural areas where electricity is not available. Since, a solar panel attached to the unit may be sufficient to carry out the process.

2.5.4.2 Disadvantages of EC

1. The ‘sacrificial electrode’ slowly carrode and are dissolved into wastewater streams as a result of oxidation and need to be regularly replaced.

2. An impermeable oxide film may be formed on the cathode leading to loss of efficiency of the EC unit.

3. The use of electricity may be expensive in many place.

4. High conductivity of the liquid suspension is required. This often makes it necessary to add a small amount of supportive electrolyte (a salt) to increase conductivity.

5. In large scale, the hydrogen gas produced may be a cause of fire hazard. The high current used may constitute a considerable degree of risk.

6. Gelatinous hydroxide may tend to solubilize in some cases.

There are many reports of electrocoagulation for natural compounds isolation.

Many years ago, Mosa and Basu[23] isolated the medicinally important alkaloids which are reserpine and ajmaline from root of *Rauvolfia serpentina* by conventional comparable with electrocoagulation technique. In EC process, root were extracted with ethanol and diluted with a small amount of water before subjecting it into EC cell for 2 hours. the results showed the extract containing two alkaloids in the same amounts as obtained in conventional technique.

In 1998, Phutthawong and Buddhasukh[28] used electrocoagulation technique to isolate glycyrrhizic acid from leaves of *Glycyrrhiza radix*. This glycoside was isolated by using EC process as the main purification step. And then, pure compound was obtain in 2-5% yield after 2.5 hours of double electrolysis and recrystallisation of the residue from the electrolysed solution.

In next year, Plumbagin was obtain from root of *Plumbago rosea* by Philip and co-worker[26]. It is also a phenolic compound with one phenolic hydroxyl group. In EC technique, a double electrocoagulation(2.5 hours) of ethanolic extract with aluminum electrodes and a little salt for supporting electrode to gave pure compound in 0.5% yield that was same yield when used other isolation methods involving organic solvent extraction and chromatographic purification.

Three pentacyclic triterpenoid compounds, namely lupeol, betulin and tetulinic acid were isolated from the bark of *Lithocarpus elegans* by Jumpatong and co-worker[27] in 2005. This study showed the electrocoagulation technique , mainly used in

removing unwanted species from wastewater, can use as a method of fractionation and isolation of natural product.

And then, Prapalert[25] used electrocoagulation technique for isolated catechin which is antioxidising components from bark of *Terminalia alata* at 5,10, 20 and 30 minutes were compared. After purification of this compound, at 20 minute of electrolysis time showed the most antioxidising activity with IC_{50} values of 6.35 mg/L.

In 2007, eight alkaloids from six plants were isolated by electrocoagulation in comparision with conventional technique by Phutthawong and co-worker[24]. The results showed that electrocoagulation technique gave lower yields for three alkaloids, equal yields for three alkaloids and higher yields for two alkaloids. In same year, Altay and co-worker[25] applied electrocoagulation technique to isolation of seven known alkaloids from red chilli.

Finally, Udomkan[32] used electrocoagulation process for isolated (11Z)-1',2'-didehydrostemofoline from root of *stemona sp.* with the percentage yield of 10.02%. Comparing to solvent extraction technique, the same alkaloid was isolated with the percentage yield of 1.78%.

2.6 Biological activities

2.6.1 Acetylcholinesterase inhibitory activity

Acetylcholinesterase inhibitors (AChEI) are used as pharmaceuticals and as pesticides, especially against insects and other arthropod vertebrates. In medicine, AChEI are employed mostly for correcting the effects of insufficient levels of ACh. Inhibition of AChE serves as a strategy for the treatment of Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease[33].

Several investigation of AChEIs assay to find a new inhibitor is important such as assay base on colorimetric methods, assay base on fluorimetric methods, assay base

on a radiometric method and assay base on mass spectrometric detection. Assay base on colorimetric methods is suitable for screening natural product compounds can inhibit acetylcholinesteras. These assays is TLC assay divided into 2 methods; Ellman's method and TLC bioautographic assay. In this study TLC bioautographic assay was focused.

TLC bioautographic assay[34]

TLC bioautography is suitable for small amount of sample. This method is based on the reaction of AChE with a-naphthyl acetate and the subsequent formation of a purple dye with a-naphthol and Fast Blue Salt B as shown in Figure 2.14. The samples are spotted on the plate before standard development. An enzyme solution is sprayed first on the plate. It is then incubated at 37°C for 20 min before spraying a mixture of a-naphthyl acetate and Fast Blue Salt B. A purple coloration appears after 1–2 min and the inhibitory compounds are shown by white spots on the plate. The inhibition is easier to see with this method given that the contrast with the background is stronger than when using Ellman's method.

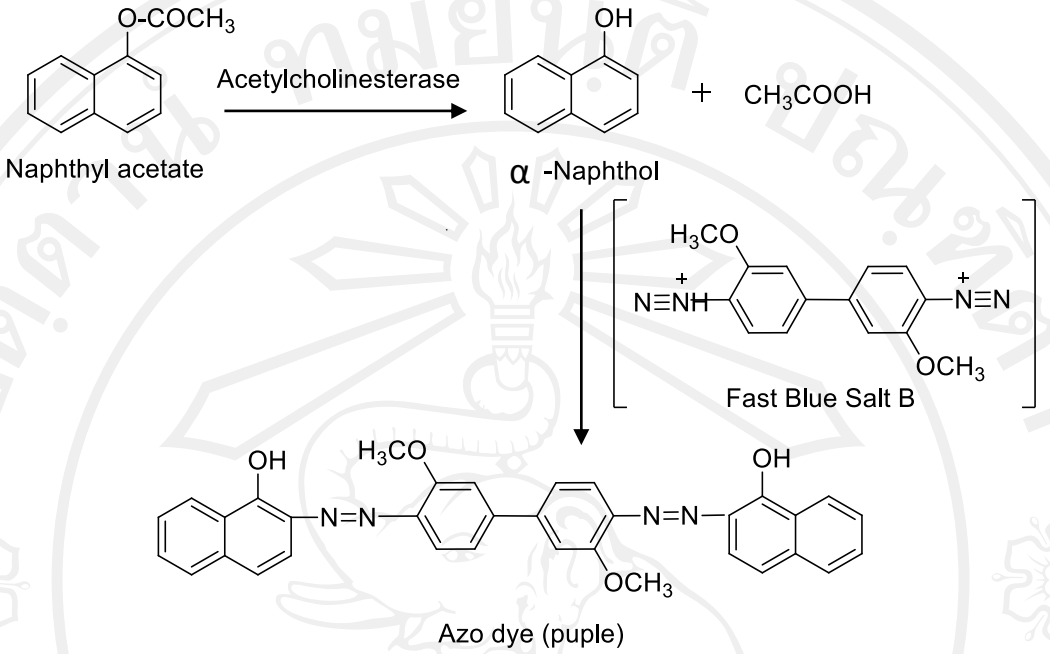


Figure 2.14 Reaction of acetylcholinesterase with naphthyl acetate and the subsequent formation of the purple dye in the TLC bioassay[34]

2.6.2 Brine shrimp lethality test (BST)[35]

The study of bioactive compounds from plant sources, extracts and isolated compounds in the chemical laboratory is often hampered by lack of suitable, simple and rapid screening procedure. They are an excellent choice for preliminary assessment of toxicity of herbal drugs/ consumer products. Brine shrimp, *Artemia* species, also known as sea monkeys, are marine invertebrates about 1 mm in size. Freeze-dried cysts are readily available at aquarium stores. The cysts last for several years and can be hatched without special equipment.