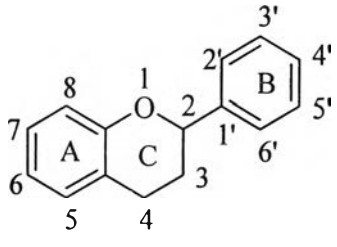
CHAPTER I

INTRODUCTION

Flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8000 individual compounds known [1]. The basic structure is the flavan nucleus consisting of 15 carbon atoms arranged in three rings (C6-C3-C6) which are labeled as A, B and C.



Basic Flavonoid Structure

Flavonoids can be divided into several categories based on hydroxylation of the flovonoid nucleus as well as the linked sugar. Essential flavonoid structures divided into eleven classes: flavone, flavonol, flavanone, flavanonol, flavan, isoflavone, chalcone, dihydrochalcone, aurone, anthocyanidin and catechin [2]. They are also important for the normal growth, development and defense of plants [3]. Flovonoids, important constituents of the human diet, are found in fruits (in citrus fruit they may represent up to 1% of fresh material) and vegetables. Beverages like red wine, tea, coffee and beer also contain large amounts of flavonoids: on the average, the daily diet contains approximately 1 g of flavonoid per day [4]. Furthermore, they are also found in several medicinal plants and herbal remedies containing flavonoids have been used in folk medicine around the world. Therefore, these compounds are important not only for plants, but also for animals, including humans.

The great prevalence of flavonoids and anthocyanidines in the vegetal kingdom is not accidental; not only do they act as the colored pigments of flowers but also as enzyme inhibitors, precursors of toxic substances, a defence against ultraviolet radiation exposure, chelating agents of metals noxious for plants, and reducing agents.

In addition, flavonoids are involved in photosensitization and energy transfer, morphogenesis and sex determination, levels of respiration and photosynthesis, action of plant growth hormones and regulators [5-9], gene expression and behavior. Though the food chain animals and humans ingest flovonoids, there is much data concerning a wide range of biological activities of these compounds in humans. For example, they were utilized in medicine as protection for vascular integrity [10], as antiosteoporotic agents [11] and for their antihepatotoxic properties [12]. Some flavonoids were examined for their activity in experimental tumor model systems both *in vitro* [13] and *in vivo* [14]. Certain flavonoids were shown to inhibit the activity of enzymes such as aldosoreductase [15] and xanthine-oxidase [16]. They were also reported to act in the gastrointestinal tract as either antiulcer [17], antispasmodic [18, 19], antisecretory or antidiarrhoel [20] agents. In addition, they were found to posses a good anti-inflammatory activity [16, 21], which this was related mainly to their inhibiting production of inflammatory mediators such as prostaglandins, leukotrienes [22]. Moreover, flavonoids were also reported as antiviral [23] antimicrobial [24, 25] antifungal [26] antitumor [27] anticancer [28] anti-HIV-1 [29] antiallergic [30] and antioxidant [31-33]. In addition, they showed agricultural activity such as antifeedant [34-37] and insecticidal activity [38].

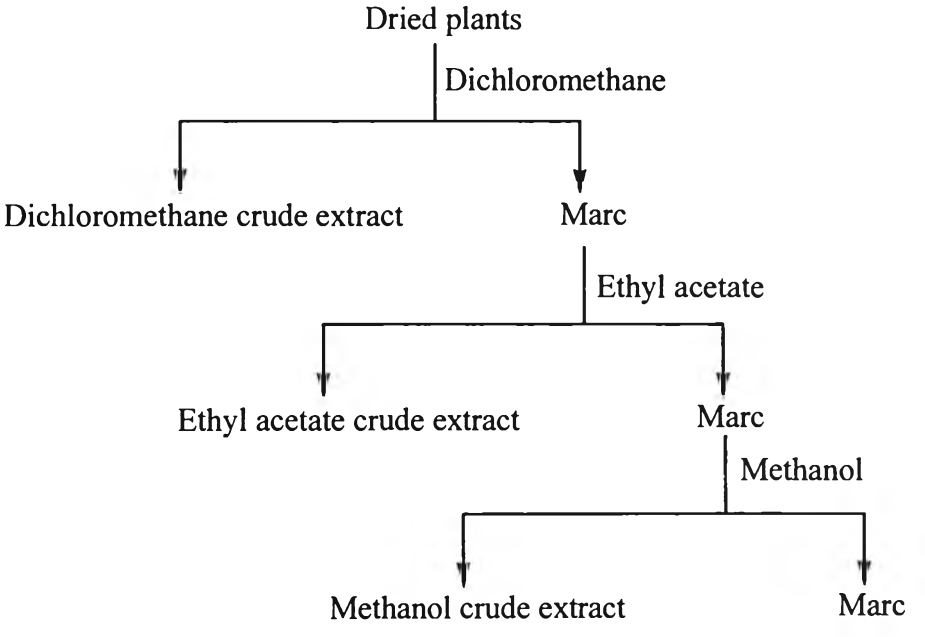
As mentioned above, flavonoids exhibited interesting biological activities and extremely important to plants and humans. In addition, many reports affirmed that flavonoids especially isoflavonoids have been isolated from plants in Leguminosae [39, 40]. Thereby, this research is focused on searching for bioactive compounds from certain plants in Leguminosae. Furthermore, some selected flavonoids are synthesized based on the fact that some natural flavonoids have a limit quantity; they are consequently not enough for further study, particularly to consider for their utilization.

1.1 Preliminary Screening Study

1.1.1 Extraction of Selected Plants

Four plants in Leguminosae; *Phaseolus lathyroides* Linn, *Mimosa pigra* Linn, *Aeschynomene americana* Linn and *Dalbergia oliveri* Gamble; were accumulated for preliminarily biological screening test. The dried crush of each plant was extracted with dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and methanol

(MeOH), respectively by soxhlet extraction. The scheme for the general extraction is shown in Scheme 1.1.



Scheme 1.1 Extraction procedures for preliminary screening test

The results of extraction are presented in Table 1.1

Table 1.1 The results of extraction of selected four plants in Leguminosae family

Plants	Part	Weight of dried plants (g)	Crude extract (g) (% wt by wt)		
			CH_2Cl_2	EtOAc	MeOH
Phaseolus lathyroides Linn	Leave	100	5.15 (5.15)	1.02 (1.02)	16.77 (16.77)
	Stem	100	1.90 (1.90)	0.19 (0.19)	5.17 (5.17)
	Root	100	1.31 (1.31)	0.29 (0.29)	3.60 (3.60)
	Seed	400	3.02 (0.76)	0.34 (0.09)	19.91 (4.98)
Mimosa pigra Linn	Leave	100	3.50 (3.50)	1.44 (1.44)	11.50 (11.50)
	Stem	100	0.38 (0.38)	0.47 (0.47)	2.20 (2.20)
	Pod	100	0.96 (0.96)	0.16 (0.16)	8.02 (8.02)
	Seed	300	5.27 (1.76)	0.22 (0.07)	11.02 (3.67)
Aeschynomene americana Linn	Leave	60	2.52 (4.20)	0.85 (1.42)	12.51 (20.85)
	Stem	100	0.69 (0.69)	0.28 (0.28)	5.01 (5.01)
Dalbergia oliveri Gamble	Leave	40	0.82 (2.05)	0.46 (1.15)	1.79 (4.48)
	Heartwood	200	11.00 (5.50)	-	23.00 (11.50)
	Bark	300	1.13 (0.38)	-	64.00 (21.33)

According to the above results, the highest amount of CH_2Cl_2, EtOAc and MeOH extracts (% wt by wt) was achieved from the leaves of *P. lathyroides* and *M. pigra* and the barks of *D. oliveri*, respectively.

1.1.2 Biological Screening Assay

Two biological screening tests; insecticidal activity and scavenging effect on DPPH radical were selected for preliminary study.

1.1.2.1 Insecticidal Activity (Contact toxicity: topical application)

The common cutworms *Spodoptera litura* obtained from Department of agriculture, Ministry of agriculture and cooperatives were used as a model for meanwhile investigation.

General Procedure [41]

The samples were prepared by dissolving 5 mg of test substances in 1 mL of acetone to provide 50,000 ppm solution. Common cutworms, *Spodoptera litura*, were reared on an artificial diet in a controlled environment. Ten of third instar larvae were placed in petri dish and 10 μL of tested compound was dropped with a micropipette. The artificial diets were contained in petri dish and kept at 25°C for 24 h (4 replications). The control solution was prepared by using only a proper solvent. After 24 h, the died cutworms were counted and converted to percentage of died larvae (% mortality) of *S. litura*.

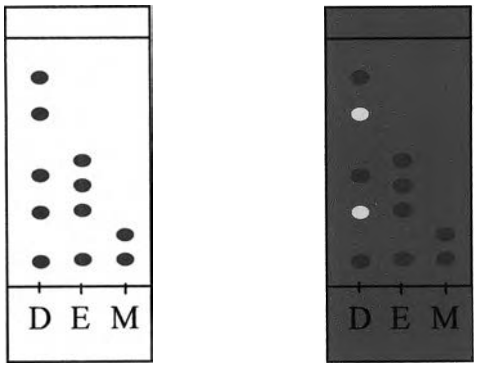
1.1.2.2 Scavenging Effect on DPPH Radical [42]

2,2-Diphenyl-1-picryhydrazyl (DPPH) radical is a stable radical with a purple color (λ_{max} 517 nm). Upon reduction by a scavenger, the extensive conjugation is disrupted and the compound turns yellow.

TLC Autographic Assay

After developing and drying, TLC plates were sprayed with 0.2% DPPH in methanol solution. The plates were observed within 10 minutes after spraying. Active compounds were visualized as yellow spot against purple background.

The chromatograms of all extracts before and after spraying with DPPH radical reagent are demonstrated in Figure 1.1.



A B

Figure 1.1 TLC autographic assay for DPPH radical scavenger
(A) TLC chromatogram before spraying with DPPH reagent
(B) TLC chromatogram after spraying with DPPH reagent
D: Dichloromethane, E: Ethyl acetate and M: Methanol

1.1.3 Results of Biological Activity Screening Test

Dichloromethane, ethyl acetate and methanol crude extracts of four selected plants were preliminarily screened for their insecticidal activity against *S. litura* and radical scavenging effect on DPPH radical by TLC autographic assay. The results are demonstrated in Table 1.2.

Table 1.2 Biological activity screening test results of crude extract

Plant	Part	Crude extact	Insecticidal activity (% mortality)	Radical savenging effect on DPPH radical
Phaseolus lathyroides Linn	Leave	CH_2Cl_2	55.0	-
		EtOAc	72.5	-
		MeOH	62.5	-
	Stem	CH_2Cl_2	55.0	-
		EtOAc	45.0	+
		MeOH	35.0	+
	Root	CH_2Cl_2	35.0	++
		EtOAc	10.0	-
		MeOH	50.0	-
	Seed	CH_2Cl_2	72.5	+
		EtOAc	70.0	+
		MeOH	67.5	-
Mimosa pigra Linn	Leave	CH_2Cl_2	50.0	-
		EtOAc	50.0	+
		MeOH	30.0	+
	Stem	CH_2Cl_2	37.5	-
		EtOAc	60.0	+
		MeOH	27.5	+
	Pod	CH_2Cl_2	12.5	-
		EtOAc	27.5	-
		MeOH	20.0	+
	Seed	CH_2Cl_2	37.5	-
		EtOAc	25.0	-
		MeOH	7.5	+
Aeschynomene americana Linn	Leave	CH_2Cl_2	40.0	-
		EtOAc	37.5	-
		MeOH	20.0	-

Table 1.2 (continued)

Plant	Part	Crude extact	Insecticidal activity (% mortality)	Radical savenging effect on DPPH radical
Aeschynomene americana Linn	Stem	CH_2Cl_2	52.5	-
		EtOAc	20.0	-
		MeOH	10.0	-
Dalbergia oliveri Gamble	Leave	CH_2Cl_2	55.0	-
		EtOAc	47.5	-
		MeOH	10.0	-
	Heartwood	CH_2Cl_2	10.0	+++
		MeOH	7.5	+++
	Bark	CH_2Cl_2	32.5	+++
		MeOH	7.5	+++

Note: + positive results observed after 10 minutes, ++ positive results observed after 5 minutes
+++ positive immediately

From these preliminary results for biological activities it was found that the ethyl acetate extract of the leaves and both dichloromethane and ethyl acetate extracts of the seed of *P. lathyroides* disclosed good insecticidal activity against *S. litura* (≥ 70% mortality). Moreover, both dichloromethane and methanol extracts of the heartwoods and the barks of *D. oliveri* revealed the excellent radial scavenging effect on DPPH radical. Therefore, in this research the heartwoods of *D. oliveri* were selected to study since its extracts displayed excellent radial scavenging effect on DPPH radical. In addition, there is only one report concerning the chemical constituents of the heartwoods of this certain species [43] while their biological activities have not been reported.

1.2 The Goal of This Research

The attractive preliminary results for bioassay of *D. oliveri* extract call for intensive investigation. Thereby, the goal of this research can be summarized as:

1. To extract and isolate the chemical constituents from the heartwoods of *D. oliveri*
2. To elucidate the structures of isolated compounds
3. To investigate biological activity of isolated compounds
4. To synthesize some selected flavonoids
5. To explore biological activity of the synthesized flavonoids