APPENDIX

APPENDIX A
MEDIA PREPARATION

Table 1 The composition of Murashige and Skoog (1962) medium

Component	Milligram/litre
Macronutrients	
NH_4NO_3	1,650
KNO_3	1,900
$CaCl_2.2H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
Micronutrients	
H_3BO_3	6.2000
$MnSO_4.4H_2O$	22.3000
$ZnSO_4.7H_2O$	8.6000
KI	0.8300
$Na_2MoO_4.2H_2O$	0.2500
$CuSO_4.5H_2O$	0.0250
$CoCl_2.6H_2O$	0.0250
Organic constituents	
Na_2-EDTA	37.25
$FeSO_4.7H_2O$	27.85
Glycine	100
Nicotinic acid	0.4
Pyridoxine-HCl	0.5
Thiamine-HCl	0.1
Sucrose	30,000
Agar	7,000
pH	5.7

Table 2 MS medium stock solution

	Chemical constituents	Concentration (g/l)
	MS-major salts	
Stock I (50x)	KNO_3	95
(use 20 ml/l)	$NH4NO_3$	82.5
	$CaCl_2.2H_2O$	22
	MS-minor salts	
	H_3BO_3	0.62
Stock II (100x)	KH_2PO	17
(use 10 ml/l)	KI	0.083
	$Na_2MoO_4.2H_2O$	0.025
	$CoCl_2.6H_2O$	0.0025
	Sulfate Stock	
	$MgSO_4.7H_2O$	37
Stock III (100x)	$MnSO_4.H_2O$	1.69
(use 10 ml/l)	$ZnSO_4.7H_2O$	0.86
	$CuSO_4.5H_2O$	0.0025
	EDTA sodium ferric salt	
Stock IV (100x)	$Na_2EDTA.2H_2O$	3.725
(use 10 ml/l)	$FeSO_4.7H_2O$	2.785
	MS-vitamins	
	Glycine	0.2
Stock V (100x)	Nicotinic acid	0.05
(use 10 ml/l)	Thiamine-HCl (B1)	0.01
	Pyridoxine-HCl (B6)	0.05
	myo-Inositol	10

Preparation of Murashige & Skoog (MS) stocks

The formulation of Murashige & Skoog's (1962) medium is given in Table 3. Additional formulations are given in Table 1.

MS-major salts stock (50x; Table 3, Stock I). Add approximately 400 ml Reverse Osmosis (R.O.) water to a 1-litre beaker. Weigh and dissolve each of the salts given in the third column using a magnetic stirrer. Transfer the solution to a 1-litre volumetric flask, and add R.O. water to the final volume. Store under refrigeration. Pipette 20 ml of the major salts stock for 1 liter of MS nutrient medium.

MS-minor salts stock (100x; Table 3, Stock II). Add approximately 400 ml R.O. water to a 1-litre beaker. Weigh and dissolve each of the salts given in the third column using a magnetic stirrer. Transfer the solution to a 1-litre volumetric flask, and add R.O. water to the final volume. Store under refrigeration. Pipette 10 ml of the minor salts stock for 1 liter of MS nutrient medium.

MS-sulfate stock (100x; Table 3, Stock III). Add approximately 400 ml R.O. water to a 1-litre beaker. Weigh and dissolve each of the salts given in the third column using a magnetic stirrer. Transfer the solution to a 1-litre volumetric flask, and add R.O. water to the final volume. Store under refrigeration. Pipette 10 ml of the sulfate stock for 1 liter of MS nutrient medium.

MS-EDTA sodium ferric salt stock (100x; Table 3, Stock IV). Dissolve FeSO4.7H2O in 40 ml of warm R.O. water in a 100-cm3 beaker. In a separate beaker dissolve Na2EDTA.2H2O in 40 ml of warm R.O. water. Mix the two solutions and transfer to a 1-litre volumetric flask. Add R.O. water to the final volume. The iron stock should be protected from light by storing the solution in an amber bottle, or wrap the entire flask with aluminum foil. Store under refrigeration. Pipette 10 ml of the EDTA sodium ferric salt stock for 1 liter of MS nutrient medium.

MS-vitamins stock(100x; Table 3, Stock V). Add about 400 ml R.O. water to a 1-litre beaker. Weigh and dissolve each of the salts given in the third column using a magnetic stirrer. Transfer the solution to a 1-litre volumetric flask, and add R.O. water to the final volume. Store under refrigeration. Pipette 10 ml of the sulfate stock for 1 liter of MS nutrient medium.

Note: Do not pipette directly from stock bottles, and do not return any unused stock solutions to the stock bottles. Label all stock solutions and include the concentration, your initials, and the date of preparation. Although inorganic salts are relatively stable in solution under refrigeration, vitamin stock should be discarded after 30 days. Also, vitamin stock should be visually examined periodically for any signs of microorganisms.

Preparation of the complete MS medium

1. Add approximately 400 ml R.O. water to a 1-litre beaker.

2. Pipette each of the MS stock solutions: 20 ml stock I, 10 ml stock II, 10 ml stock III, 10 ml stock IV, 10 ml stock vitamins. Mix it using a magnetic stirrer.

3. Weigh 30 g sucrose and dissolve it in the medium mixture.

4. Add R.O. water until the total volume of liquid is about 800 cm3. While agitating the solution with a magnetic stirrer, adjust the pH to 5.7 with droplets of 1N NaOH or 1N HCl with separate Pasteur pipettes.

5. Transfer the medium to a 1-litre volumetric flask and add R.O. water to the final volume.

6. Weigh 0.245 g Phytagel™ and dissolve it in the medium mixture using a magnetic stirrer. Cover beaker with clear film protecting volatilization.

7. Boil in a microwave for 10 minutes. Each of wide necked bottle with fitted lids sized 8 Onz. can be poured with 30 ml medium and then autoclave at 121 °C for 20 min.

8. After the sterilized medium is removed from the autoclave, the bottles are swirled for a few minutes to ensure the dissolution of the medium. After the gel in the bottles has cooled, store in clean cabinet.

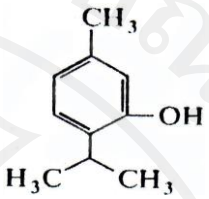
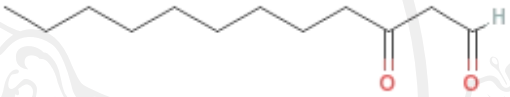
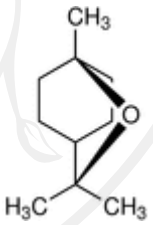
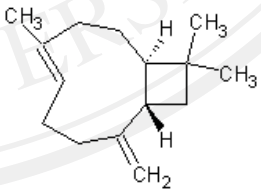
APPENDIX B
MOLECULAR STRUCTURE OF ACTIVE INGREDIENTS FROM PLOOKAO

We have found 6 important different kinds Chemical composition.

1. Volatile oil

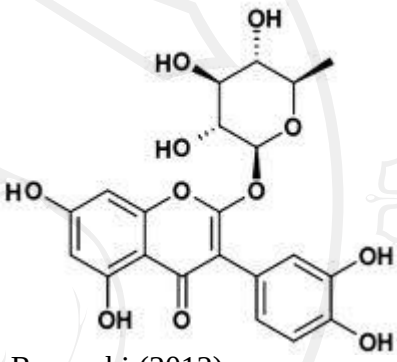
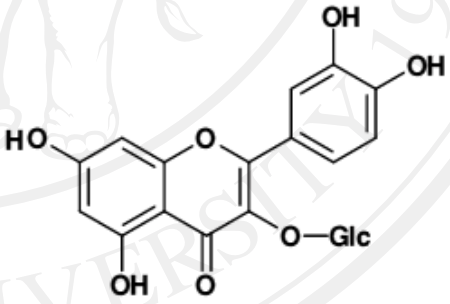
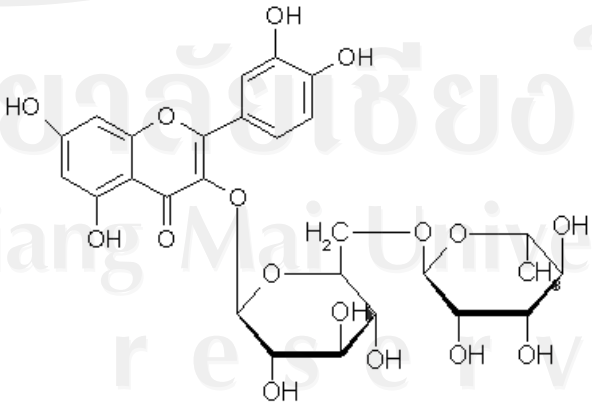
According to the research, we have found the Essential oil 0.005-.05 % in Plookao. And the Plookao from different places have a big range percentage of essential oil. So, in this essential oil, we have found d-bormeol; bornyl Acetate; camphene; capryl aldehyde; α-caryophyllene; caryophyllene oxide; 1,8cineol; *p*-cymene; decan-1-al; decanoate methyl; 3,5-didecanoyl-4-nonyl; 3-deanoyl-4-nonyl-5-dodecanoyl; n-decyladehyde; 3,5-didodecaoyl-4-nonyl;dodecan1al; dodecanaldehyde; dodecanoate, methyl; geraniol; hexadecanoate, methyl-n-nonyl ketone; limonene; linalool; 2-nethythp-tenone; methyl-n-noyl ketone; myrcene; octan-1-al; α-pinene; *b*-pinene; thymol; undeca-2-one; vomifoliol (Tang and Eisenbran, 1992). Also the essential not constant type such as decanoyl acetaldehyde or 3-oxododecanal react polymerization reaction easily, so there is the synthesis became Sodium component of decanoyl acetaldehyde which named commercially Houttuynuim. There are both tablet and for injection or hydrozine in component synthesis of nicotine acid to make a medicine for anti bacteria especially for lungs infected tuberculosis (Tang, W. and Eisenbrand G., 1992). Besides this, we have found the essential oil from Plookao can anti Herpies Symplexs and also HIV virus. See Table 3.

Table 3 The chemical structure of some volatile oil that found in Plookao

Active Ingredients	Structure
Thymol	 Source : Wikipedia (2013)
Decanoyleacetaldehyde	 Source : ChemDrug (2010)
1,8-Cimeol	 Source : wikimedia (2012)
Caryophyllene	 Source : Ganfyd (2008)

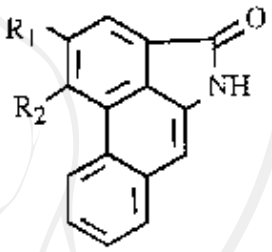
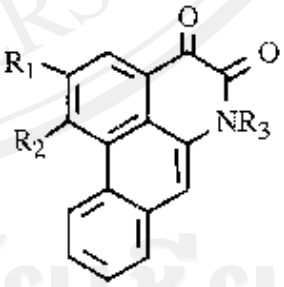
2. Flavonoids according to the report, they have found various kinds of Flavonoids and Flavonoid Glycoside such as Afzelin, Hyperin, Isoquercetin, Isoquercitrin, Quercetin, Quercitrin, Reynoutrin and Rutin. See picture

Table 4 The chemical structure of some flavonoids that found in Plookao

Active Ingredients	Structure
Quercitrin	 Source : Rozanski (2012)
Hyperin	 Source : Wildflower (No date)
Rutin	 Source : Chemweb (2003)

3. Alkaloids they have found various kinds of alkaloids for example, aristolactam A; aristolactam A II; aristolactam B; ceparanone B; cepharadione B; cordarine; norcepharadione B; piperolactam A; 1,4-dihydropyridine; 2-nonyl-5-decanoylpyridine; 3,5- didecanoylpyridine; benzamide, cis-N-(4-hydroxy-styryl); benzamide trans-N-(4-hydroxy-styryl) (Kim *et al*., 2001).

Table 5 The chemical structure of some alkaloids that found in Plookao

Active Ingredients	Structure
Alkaloids	 Cepharanone B: $R_1 = R_2 = OCH_3$ Aristolactam A; II; = OH, $R_2 = OCH_3$ Piperolactam A; $R_1 = OCH_3$, $R_2 = OH$ Source : Herbal wine (2006)
	 Cepharadione B; $R_1 = R_2 = OCH_3$, $R_3 = OCH_3$ Norcep haradione B; $R_1 = R_2 = OCH_3$, $R_3 = H$ Source : Herbal wine (2006)

4. Fatty acids

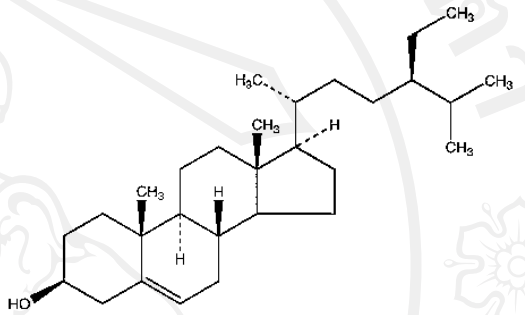
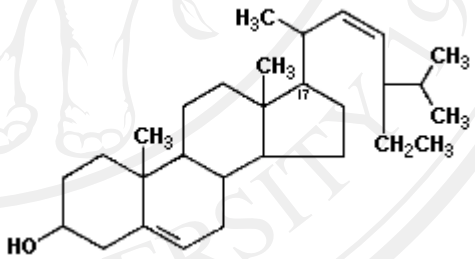
It is reported that the fatty acid is a substance found in plookao types include fixed oil, such as capric acid, lauric acid, linoleic acid, linolenic acid, palmitic acid, steric acid, tetradecanoic acid (Tang and Eisenbrand,1992).

Table 6 The chemical structure of some fatty acids that found in Plookao

Active Ingredients	Structure
Capric Acid; n = 8 Lauric Acid; n = 10 Tetradecanoic Acid; n = 14 Palmitic Acid; n = 14 Steric Acid; n = 16	$CH_3—(CH2)_n—COOH$ Source : Medicinal Plant Research Institute (2003)

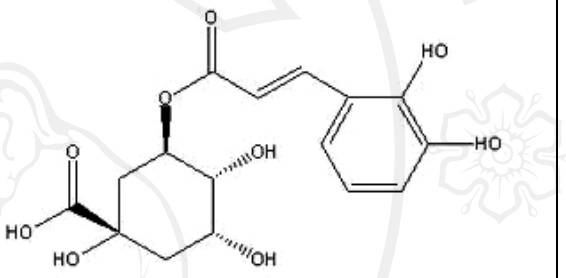
5. Sterols such as phytol; *b*-sitosterol; spinasterol; stigmasterol (Tang and Eisenbrand,1992).

Table 7 The chemical structure of some sterols that found in Plookao

Active Ingredients	Structure
B-Sitosterol	 Source : International Journal of Nutrition, Pharmacology, Neurological Diseases (2010)
Stigmasterol	 Source : Zamora (2013)

6. The other types of chemical substances that found in Plookao such as chlorogenic acid and mineral are fluoride; potassium chloride; potassium sulfate (Medicinal Plant Research Institute, 2003).

Table 8 The chemical structure of the other types of chemical substances that found in Plookao

Active Ingredients	Structure
Chlorogenic Acid	 Source : Herbal wine (2006)

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The Proceedings

[1] Nairong, N., and Wongroung, S. (2010). *In vitro* Prpagation of Plookao (*Houttuynia cordata* Thunb.). The 22nd Annual Meeting of the Thai Society for Biotechnology "TSB2010: International Conference on Biotechnology for Healthy Living" Prince of Songkla University, Trang campus, Trang, Thailand. October 20th-22nd, 2010.

The Presentations

[1] Nairong, N., and Wongroung, S. (2010). Micropropagation of Plookao (*Houttuynia cardata* Thunb.). The International Conference on Agriculture and Agro-Industry 2010 : Food Health and Trade (ICAAI2010). November 19th-20th, 2010. Mae Fah Luang University, Chiang Rai, Thailand.

[2] Nairong, N., and Wongroung, S. (2010). *In vitro* Prpagation of Plookao (*Houttuynia cordata* Thunb.). The International Conference on Biotechnology for Healthy Living 2010 (TSB2010). October 20th-22nd, 2010. Prince of Songkla University, Trang campus, Trang, Thailand.