CHAPTER IV

RESULTS

PHARMACOGNOSTIC SPECIFICATIONS

Morphological identification

Macroscopic and microscopic specifications were illustrated in figures 14-17, 21-24, 28-31, 35-38 and 42-45. Cytological and histological characterization showed a valuable tool for the identification of each ingredient in Ben-Cha-Moon-Yai remedy.

Physiochemical identification

The physico-chemical specification (% by weight) of each root species in Ben-Cha-Moon-Yai remedy was demonstrated as the grand average and pooled standard deviation in different parameters.

Thin layer chromatographic analysis

The TLC fingerprint of the methanolic extract from five root species were performed using Silica gel F_{254}. The resolution of the separation was tried in different solvent systems and the best resolving solvent system was chosen for developing the plates. The plates were visualized under UV light at 254 nm and 365 nm and were then exposed to 10% sulfuric acid reagent. The fingerprints of each root species were provided in figure 18, 25, 32, 39 and 46.

High performance liquid chromatographic analysis

To develop the representative fingerprint, the ethanolic extract of five root species and Ben-Cha-Moon-Yai remedy were analyzed under the same HPLC condition. In order to obtain the optimal elution conditions for the separation and determination of the constituents, various linear gradients of 10 mM phosphoric acid and acetonitrile at a flow rate of 0.8 ml/min were investigated. The chromatogram

revealed a common peak of each root extract and Ben-Cha-Moon-Yai remedy within the retention time of 60 min as shown in figure 19, 26, 33, 40 and 47.

Liquid Chromatography-Mass spectrometry

LC-ESI-MS method was employed to analyze the component in Ben-Cha-Moon-Yai remedy and five root species extracts. The detected chromatographic and spectrometric data of the common peak in the HPLC chromatograms were provided in figure 20, 27, 34, 41 and 48. The mobile phase consisted of (A) Water and (B) Acetonitrile with a suitable gradient. ESI in positive modes under the optimized MS condition were used to detect the chemical constituents of the extracts. In the ESI-MS experiment, the molecular weight of each separated peak was obtained.

***Aegle marmelos* (L.) Correa ex Roxb.**

Family: Rutaceae

Vernacular names

Thailand: matum, tum (Pattani), ma pin (north). Bael or bel fruit (En). Bel Indient (Fr). Indonesia: maja, maja batu. Malaysia: bilak, bila, bel. Philippines: bael. Burma: opesheet, okshit. Cambodia: bnau. Laos: toum. Vietnam: trai mam.

Distribution

Bael grows wide in dry forest in the Indian Peninsula, Sri Lanka, Pakistan and Bangladesh. It is an old cultivated tree in that region, particularly found in temple gardens in India. It has spread to Indo-China, South-East Asia (in particular Thailand, northern Malaysia, eastern Java and north Luzon) and other parts of the tropics [184].

Description

A deciduous tree, 20 to 25 feet in height and 3 to 4 feet in grinth, with straight, sharp, axillary thorns and trifoliate aromatic leaves. The flowers are greenish white. The fruits are globose, 2 to 4 inch in diameter, gray or yellowish and with smooth, hard, aromatic rind. Seeds are numerous, oblong and compressed, and the pulp is mucilaginous, thick, orange red in color. The root bark is 3.5 mm thick, curved, with its surface cream yellow or grayish in color. The surface is rough, irregular, and shallow with ridges along the line of lenticels and ruptured all over. The stem bark is externally gray and internally cream in color. The outer surface is rough and warty. It is 4 to 8 mm thick, firm in texture and occurs as flat or channeled pieces. The fracture is tough and gritty in the outer region and fibrous in the inner; taste is sweet. The root in transection shows a pentarch to heptarch stele, the cork cambium arising in the pericycle. The cork is lignified and stratified, the phelloderm is composed of a wide zone of parenchymal cells with strands of stone cells in the mature bark. The medullary group in the inner region is uni-to triseriate, while in the outer region it is bi-to pentaseriate [185].

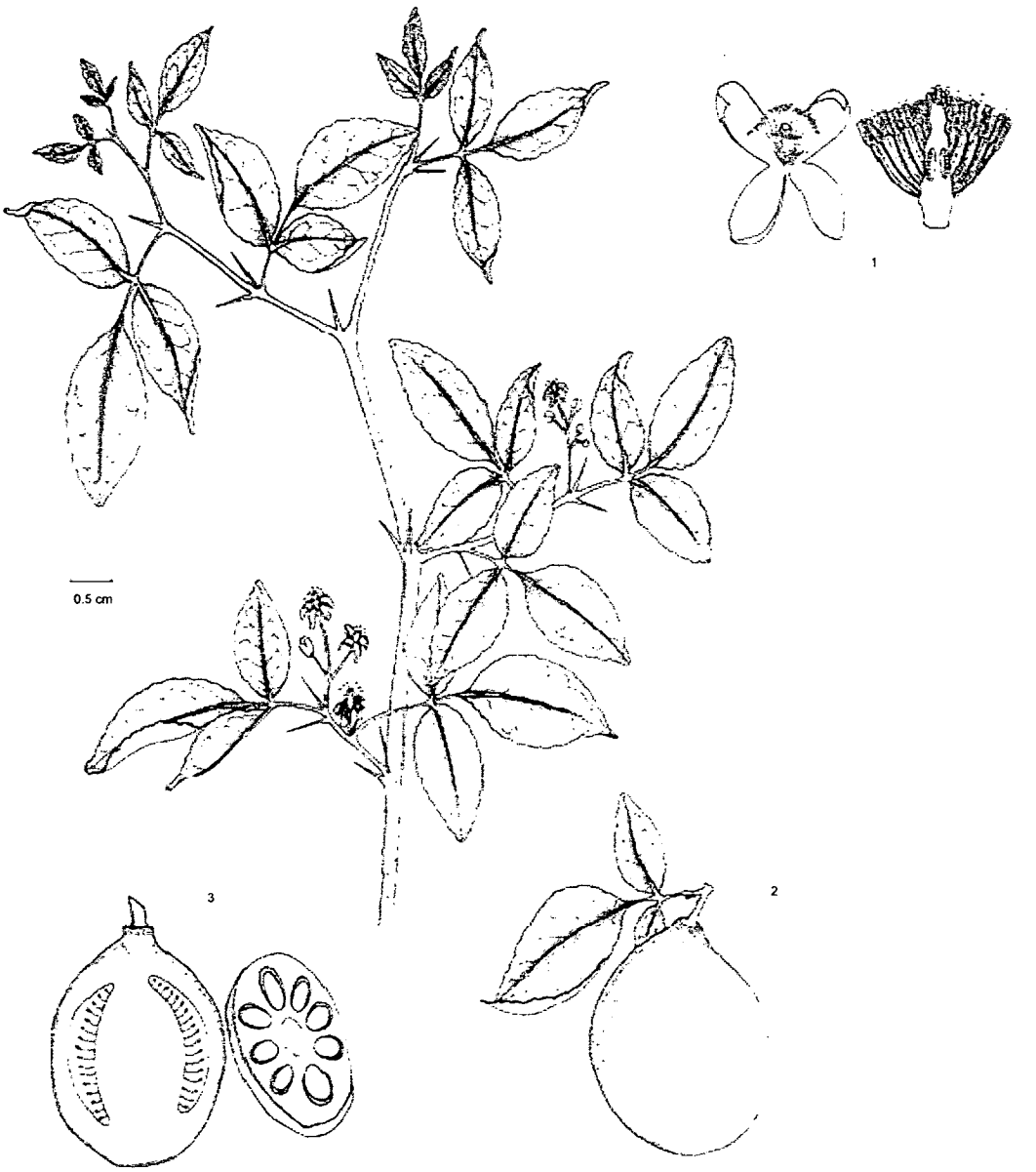


Figure 14 The flowering branch of *A. marmelos*

1. Flower 2. Fruit 3. Transverse section of fruit

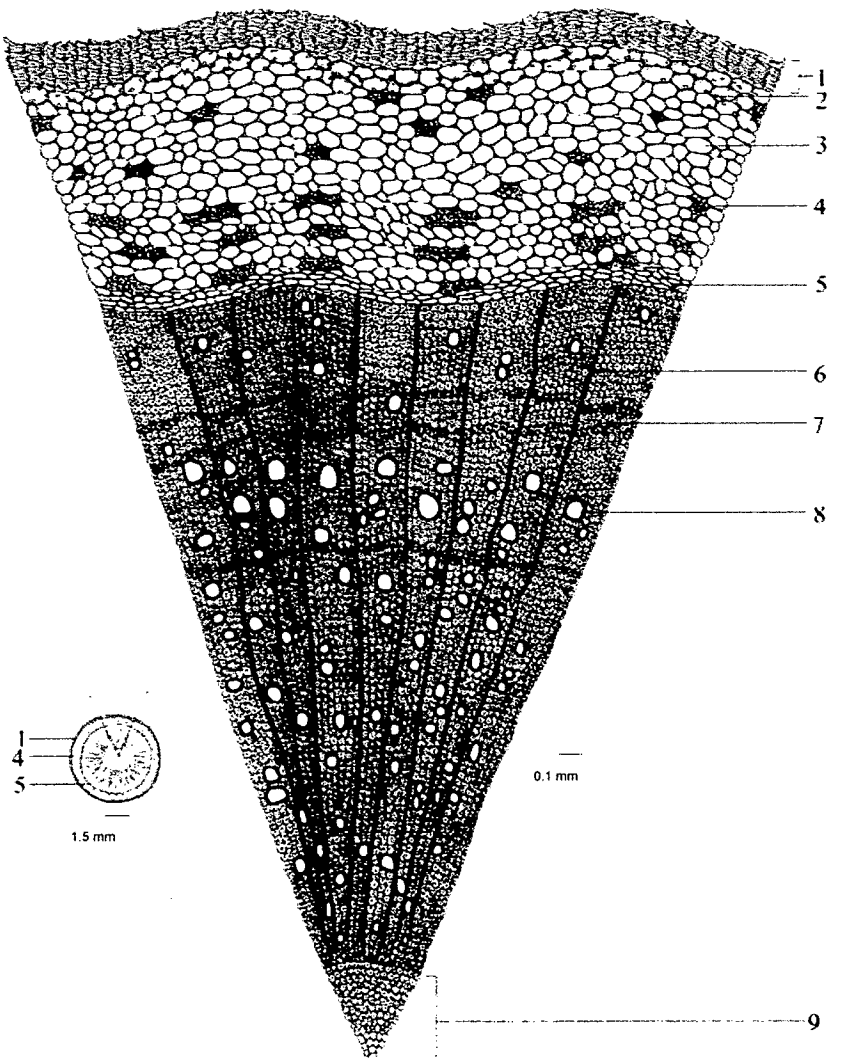


Figure 15 Transverse section of *A. marmelos* root; 1. Periderm 2. Strach granule in reserved parenchyma 3. Cortical parenchyma 4. Group of cortical fiber 5. Endodermis 6. Xylem ray with starch granule 7. Xylem fiber 8. Xylem vessel 9. Pith

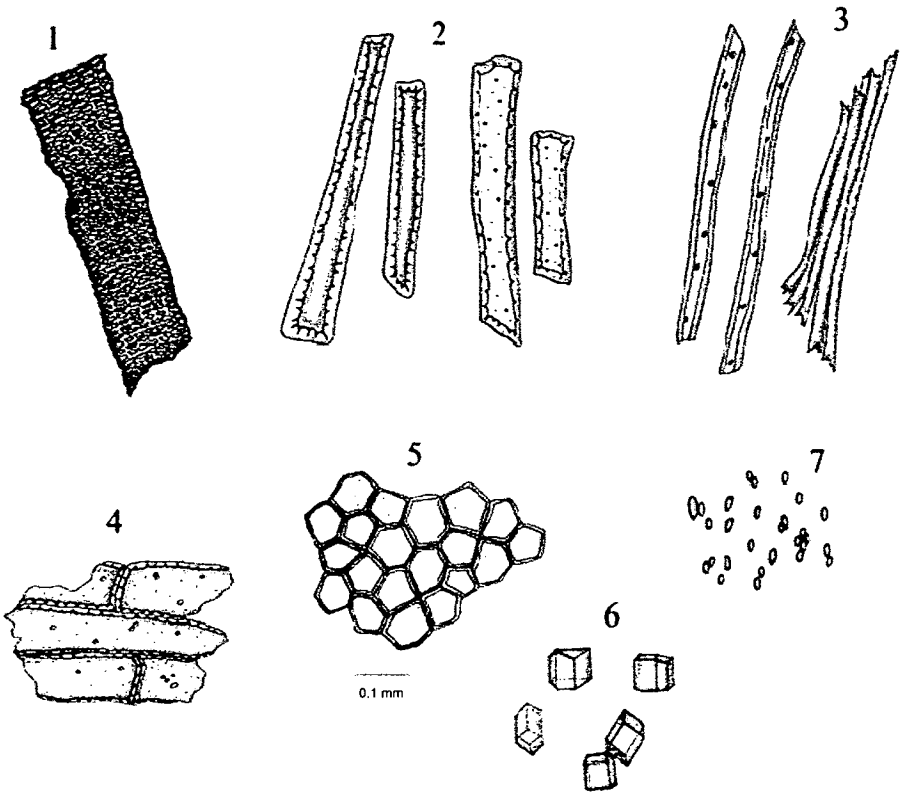


Figure 16 Powder of *A. marmelos* root ; 1. Fragment of reticulated vessel 2. Sclereid in longitudinal view 3. Fragment of fiber 4. Fragment of parenchyma in longitudinal view 5. Cork in surface view 6. Prism crystal of calcium oxalate 7. Starch granule

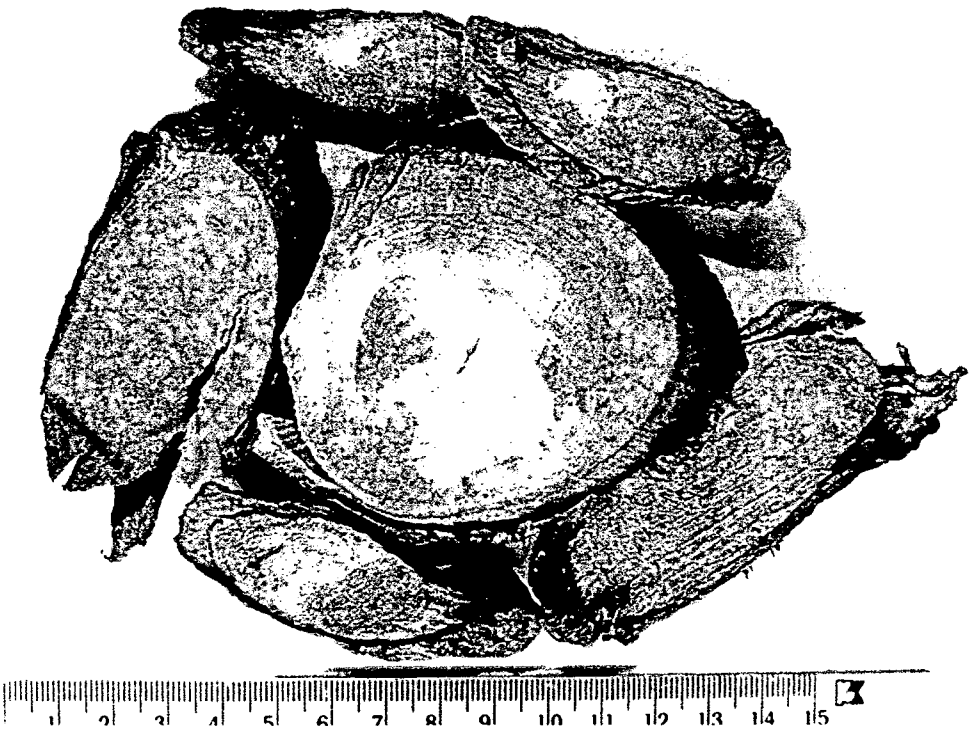


Figure 17 The root of *A. marmelos*

Table 8 Physico-chemical specification (% by weight) of *A. marmelos* root

Content (% by weight)	**Mean ±SD**	**Min-Max**	**n**
Loss on drying	6.97 ± 0.69	5.44-7.89	12
Total ash content	3.94 ± 0.89	2.56-5.49	12
Acid-insoluble ash content	0.70 ± 0.26	0.32-1.18	12
Water content	9.63 ± 1.24	7.94-11.58	12
Ethanol extractive values	5.19 ± 1.60	4.30-13.56	12
Water extractive values	6.48 ±2.42	1.64-7.50	12

Grand mean values were calculated from 12 sources throughout Thailand. Each source was performed in triplicate.

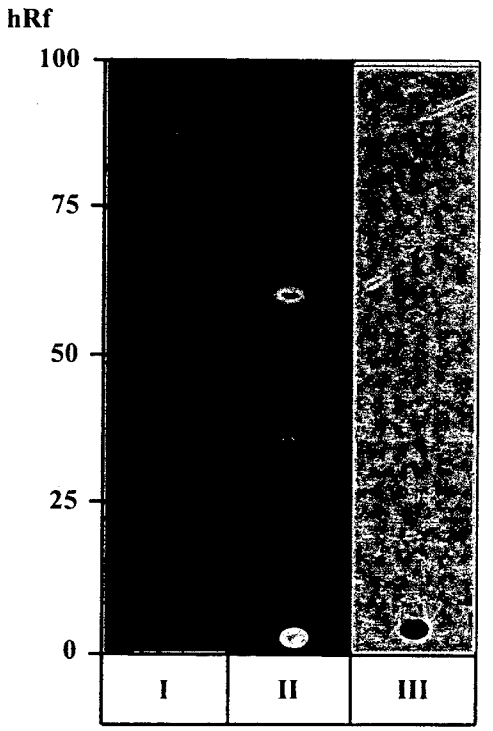


Figure 18 Thin-layer chromatogram of the methanolic extract *A. marmelos* root

Solvent system

Toluene : Ethyl acetate 3:1

Detection

I	=	detection under UV light 254 nm
II	=	detection under UV light 366 nm
III	=	detection with 10% sulfuric acid *, **

*10% sulfuric acid reagent

Preparation: conc. sulfuric acid 10 ml. in methanol 90 ml.

**Spot colour development

Heat the plate at 105 ^{o} C for 10 minutes after sprayed.

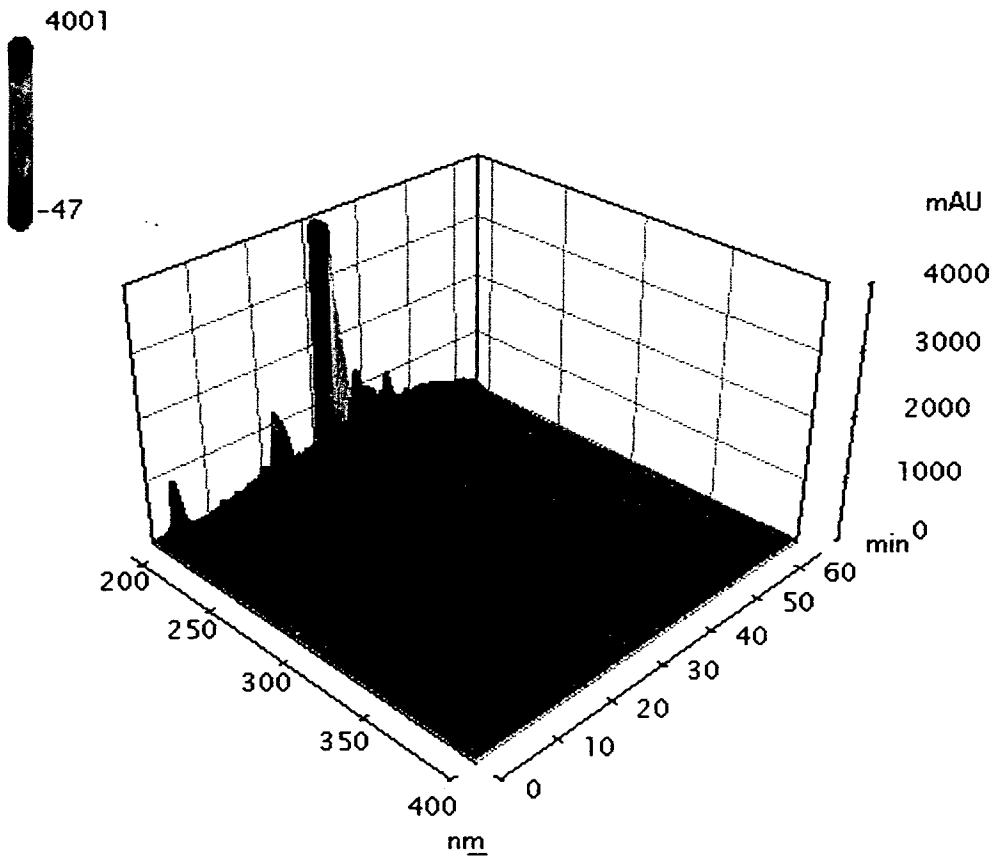


Figure 19 The 3D-HPLC profiles of ethanolic extract from *A. marmelos* root

Analysis condition

Column: Inersil ® ODS-3, C-18 column (particle size of the packing 5µm, 4.6 x 250mm)

Mobile phase: 10 mM Phosphoric acid-Acetonitrile

Linear gradient: (95:5, 65 min)

Flow rate: 0.8 mL/min

Injection volume: 10 µl

Temperature: 40°C

Wavelength: 190-400 nm.

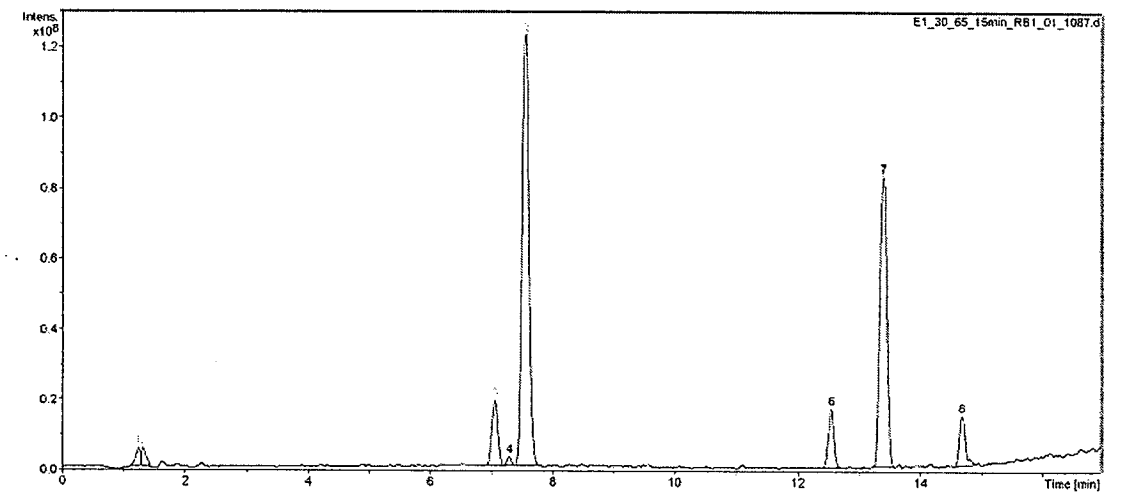


Figure 20 A representative LC/MS chromatogram from *A. marmelos* root of compound 1-8

Analysis condition

Column: Dionex C16 Acclaim RSLC PolarAdvantage column (2.1 x 100 mm, 2.2 µm,120 °A)

Mobile phase: (A) Water and (B) Acetonitrile

Linear gradient: 0-2 min (70:30%), 2-17 min (30-65%)

Flow rate: 200 µL/min

Injection volume: 5 µL of 10 ppm

Temperature: 35 °C

MS mode: UltraScan mode between m/z 70-1,500, using positive ionization

Table 9 LC- ESI-MS data and identification of constituents from the root of *A. marmelos*

Peak no.	*t*R (min)	MW	Product ions (m/z)
1	1.2	447.3	448.2, 465.1, 465.4, 470.1, 502.3
2	1.3	233.9	235.0, 252.1, 256.9, 273.0, 288.9
3	7.1	259.0	260.0, 277.1, 282.0, 297.9, 314.0
4	7.3	229.0	230.1, 247.0, 252.0, 268.0, 283.9
5	7.6	300.1	301.1, 318.0, 355.1
6	12.5	336.1	337.1
7	13.4	328.1	351.0, 383.1
8	14.7	298.1	299.1, 316.0, 321.0, 337.1, 353.0

Dimocarpus longan* Lour. Subsp. *Longan* var. *longan

Family: Sapindaceae

Synonyms

- ssp. *Longan* var. *longan*: *Dimocarpus longan* Lour. (1790), *Euphoria longana* Lamk (1792) nom. Illeg., *Nephelium longana* Cambess. (1829).
- ssp. *Longan* var. *longepetiolulatus* Leenh.: *Euphoria morigera* Gagnep. (1950) nom. Inval.
- ssp. *Longan* var. *obtusus* (Pierre) Leenh.: *Euphoria scandens* Winit & Kerr.
- ssp. *Malesianus* Leenh. Var. *malesianus*: *Nephelium malaiense* Griff. (1854), *Euphoria cinerea* Radlk. (1878) nom. Illeg., *Euphoria malaiensis* Radlk. (1879) nom. Illeq., *Euphoria gracilis* Radlk. (1913) nom. Illeg.
- ssp. *Malesianus* Leenh. Var. *echinatus* Leenh.: *Euphoria nephelioides* Radlk. (1914) nom. Illeg.

Vernacular names

- ssp. *Longan* var. *longan*: longan (En). Longanier, oeil de dragon (Fr). Indonesia, Malaysia: lengkeng. Burma: kyet mouk. Cambodia: mien. Laos: lam nhai, nam nhai. Thailand: lamyai pa. Vietnam: nhan.
- ssp. *Longan* var. *obtusus*: Thailand: lamyai khruer, lamyai tao.
- ssp. *Malesianus* var. *malesianus*: Malaysia: mata kucing (Peninsular Malaysia and Sabah), isau, sau, kakus (Sarawak). Indonesia: buku, ihau (Kalimantan), medaru (Sumatra)

Distribution

- ssp. *Longan* var. *longan*: Whereas some authors limit the area of origin to the mountain chain from Burma through southern China, others extend it to south-west India and Sri Lanka, including the lowlands. The crop is mainly grown in south China, Taiwan and north Thailand with small acreages elsewhere in Indo-China as well as Queensland (Australia) and Florida (United States) and scattered trees at higher elevations in South- East Asia.
- ssp. *Longan* var. longepetiolulatus: southern Vietnam.
- ssp. *Longan* var. *obtusus*: Indo-China, cultivated in Thailand.

- ssp. *Malesianus* var. *malesianus*: all over Indo-China and Malaysia, greatest variation found in Borneo.
- ssp. *Malesianus* var. *echinatus*: Borneo and the Philippines.

Description

Tree, up to 40 m tall and 1 m trunk diameter, sometimes buttressed, exceptionally a scandent shrub; branches terete with 5 faint grooves, sometimes warty lenticellate, rather densely ferruginous tomentose. Leaves 2-4(-6)-jugate, axial parts mostly densely hairy; petiole 1-20 cm, petiolules 0.5-35 mm long; leaflets elliptical, 3-45 cm x 1.5-20 cm, 1-5 times longer than wide, chartaceous to coriaceous, above often tomentose in basal part of midrib, beneath thinly tufted-tomentose mainly on midrib and nerves. Inflorescences usually terminal, 8-40 cm long, densely tufted-tomentose; cymules (1-)3-5-flowered; pedicels 1-4 mm; bracts patent, 1.5-5 mm long; flowers yellow-brown; calyx lobes 2-5 mm x 1-3 mm; petals 5, 1.5-6 mm x 0.6-2 mm, densely woolly to glabrous; stamens (6-)8(-10), filament 1-6 mm. Fruit drupaceous, 1-3 cm in diameter, lobe(s) broad-ellipsoid to globular, smooth to warty or sometimes up to 1 cm aculeate, sometimes granular, glabrescent, yellowbrown. Seed globular with shining blackish-brown testa; seed enveloped by a thin fleshy, translucent white arilloid [186].

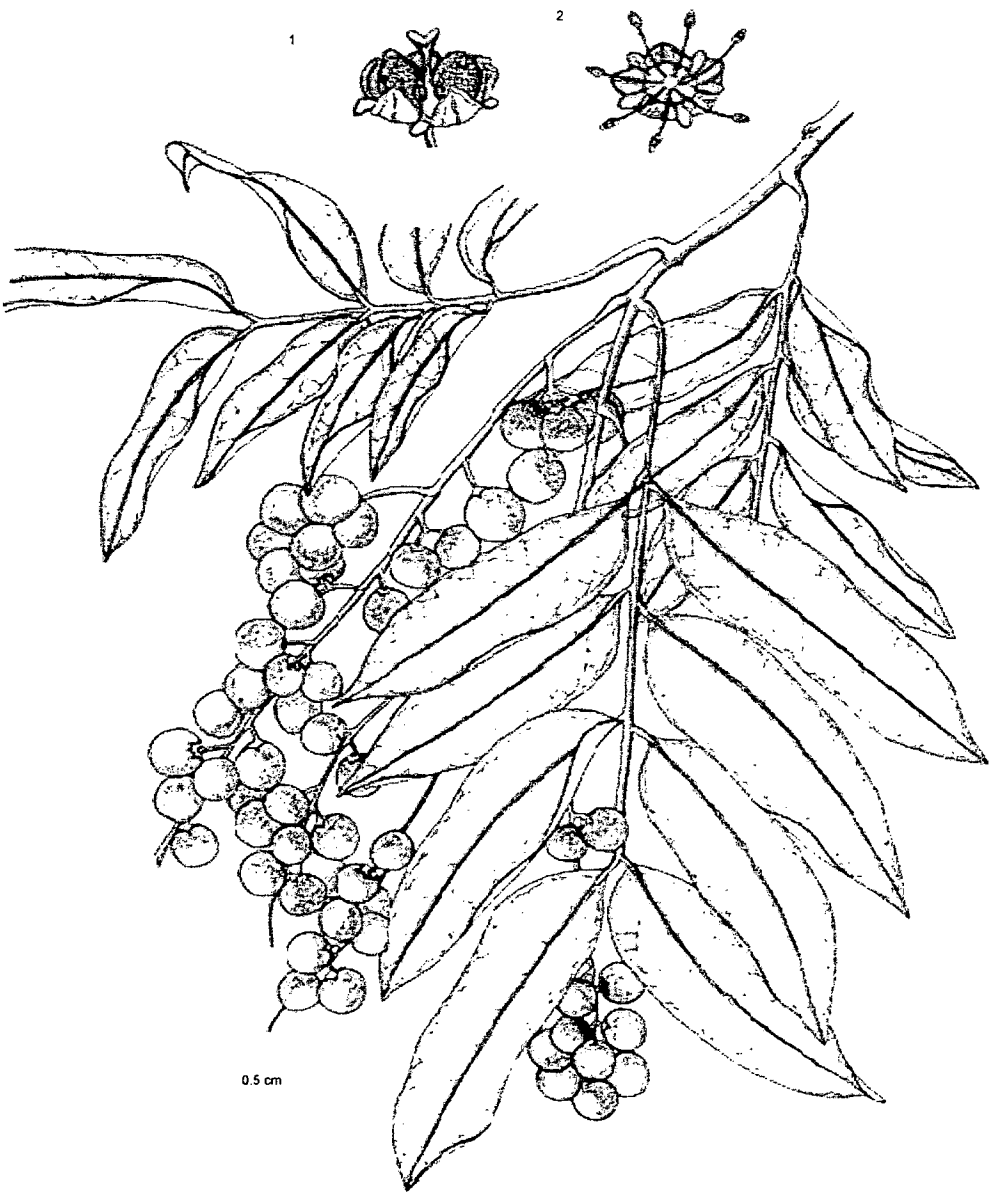


Figure 21 The fruiting branch of *D. longan*

1. Male flower 2. Female flower

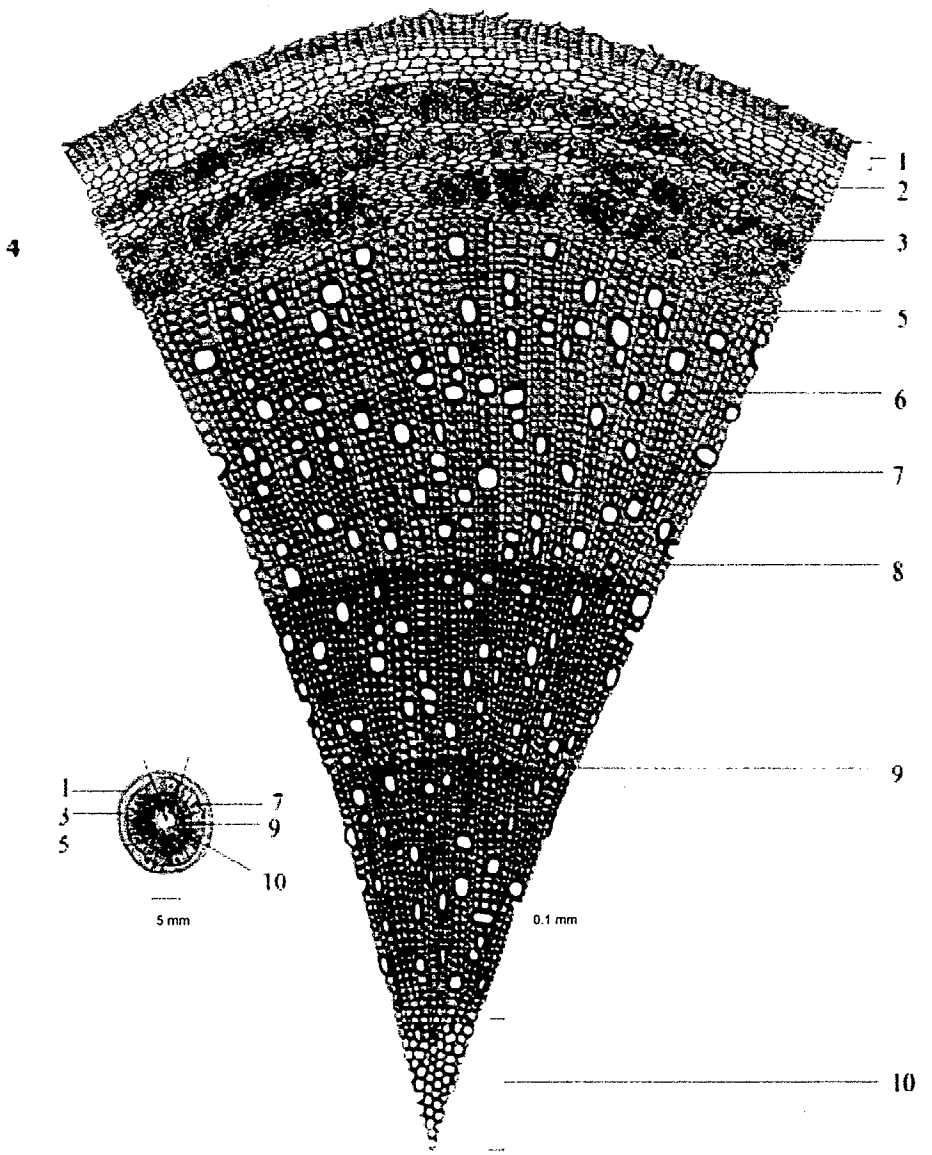


Figure 22 Transverse section of *D. longan* root ; 1. Periderm 2. Cortical parenchyma 3. Cortical fiber 4. Sclereid 5. Endodermis 6. Xylem vessel 7. Xylem ray 8. Xylem fiber 9. Annual ring 10. Pith

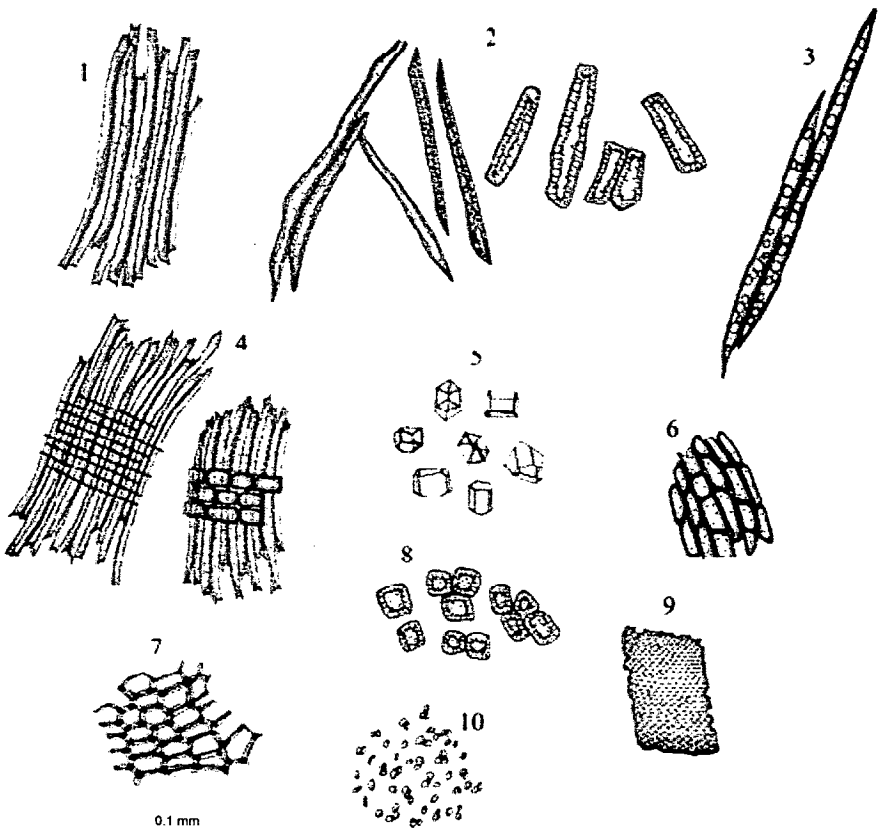


Figure 23 Powder of *D. longan* root ; 1. Fragment of fiber 2. Sclereid in longitudinal view 3. Fiber containing oil globule 4. Xylem in radial longitudinal view 5. Prism cystal of calcium oxalate 6. Parenchyma in longitudinal view 7. Cork in surface view 8. Sclereid in transverse view 9. Fragment of pitted vessel 10. Starch granule

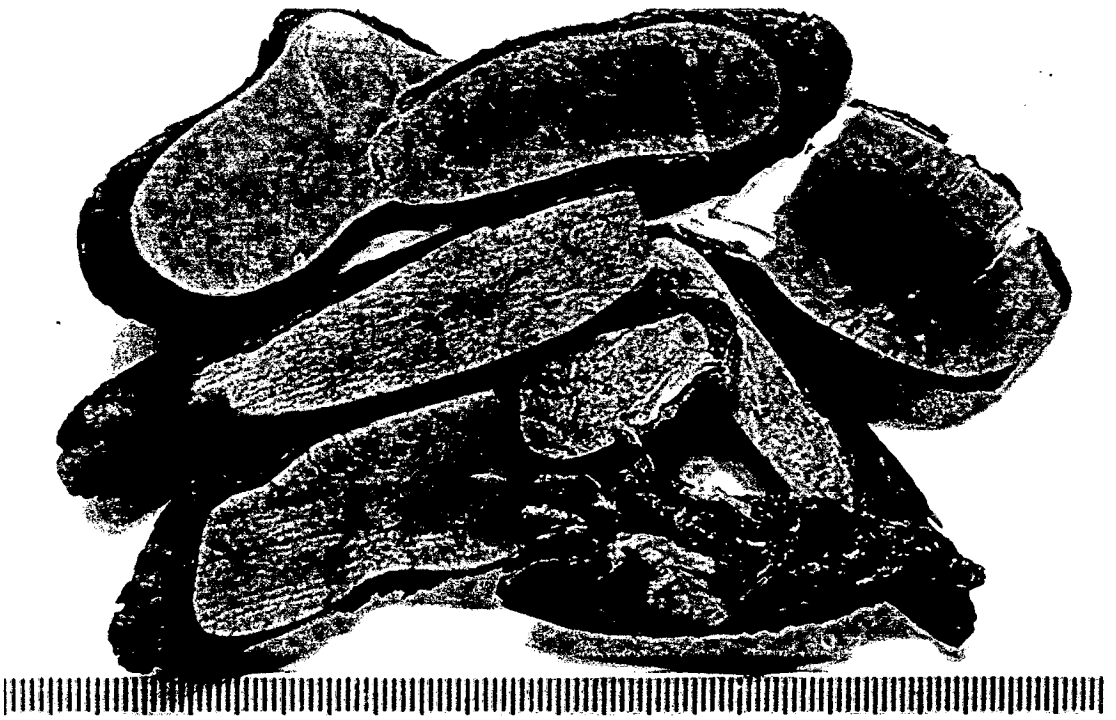


Figure 24 The root of *D. longan*

Table 10 Physico-chemical specification (% by weight) of *D. longan* root

Content (% by weight)	**Mean ±SD**	**Min-Max**	**n**
Loss on drying	7.98 ± 0.94	6.64-10.23	13
Total ash content	3.64 ± 1.43	2.02-7.15	13
Acid-insoluble ash content	1.09 ± 1.52	0.27-5.55	13
Water content	10.57 ± 1.11	8.83-12.34	13
Ethanol extractive values	7.66 ± 1.98	3.47-6.59	13
Water extractive values	5.09 ±1.05	4.53-11.00	13

Grand mean values were calculated from 13 sources throughout Thailand. Each source was performed in triplicate.

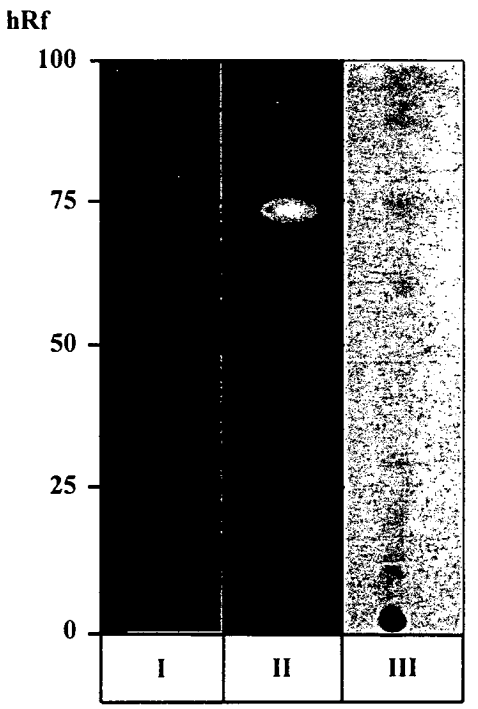


Figure 25 Thin-layer chromatogram of the methanolic extract *D. longan* root

Solvent system

Chloroform : Methanol 9 : 1

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*,**

*10% sulfuric acid reagent

Preparation: conc. Sulfuric acid 10 ml. in methanol 90 ml.

**Spot color Development

Heat the plate at 105 ° C for 10 minutes after sprayed.

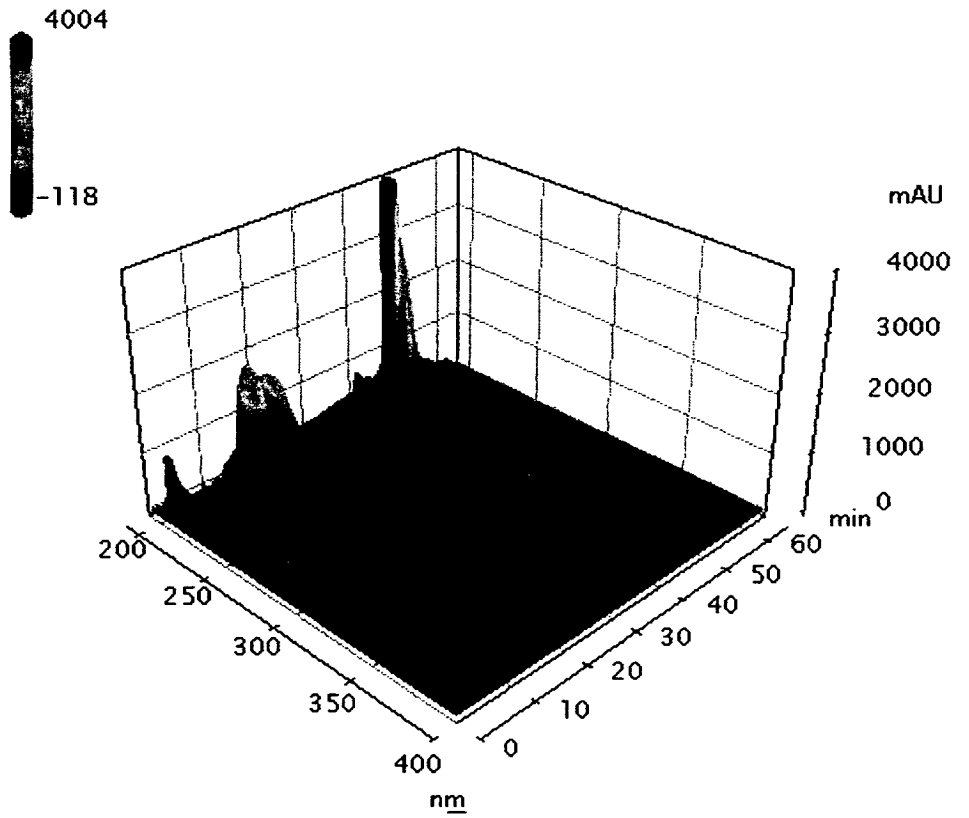


Figure 26 The 3D-HPLC profiles of ethanolic extract from *D. longan* root

Analysis condition

Column: Inersil ® ODS-3, C-18 column (particle size of the packing 5μm, 4.6 x 250mm)

Mobile phase: 10 mM Phosphoric acid-Acetonitrile

Linear gradient : (95:5, 65 min)

Flow rate : 0.8 mL/min

Injection volume : 10 μl

Temperature : 40°C

Wavelength : 190-400 nm.

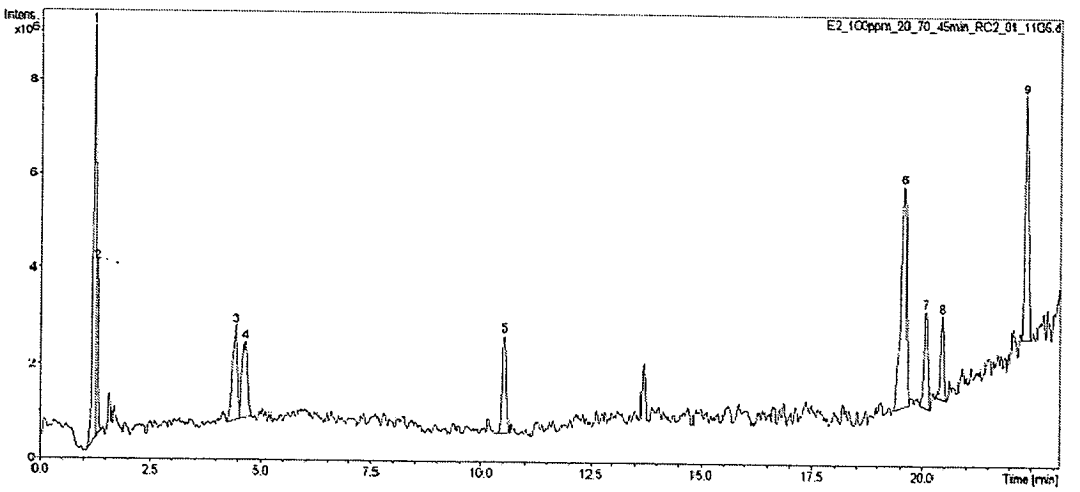


Figure 27 A representative LC/MS chromatogram from *D. longan* root of compound 1-9

Analysis condition

Column: Dionex C16 Acclaim RSLC PolarAdvantage column (2.1 x 100 mm, 2.2 μm,120 °A)

Mobile phase: (A) Water and (B) Acetonitrile

Linear gradient: 0-2 min (80:20%), 2-32 min (30-70%)

Flow rate: 200 μL/min

Injection volume: 5 μL of 100 ppm

Temperature: 35 °C

MS mode: UltraScan mode between m/z 70-1,500, using positive ionization

Table 10 LC- ESI-MS data and identification of constituents from the root of *D. longan*

Peak no.	*t*R (min)	MW	Product ions (m/z)
1	1.21	342.02	360.15, 365.01, 380.98, 397.02
2	1.28	252.06	253.13, 270.09, 275.05, 290.97
3	4.43	450.05	451.28, 489.10, 505.06
4	4.63	420.03	421.00, 438.22, 475.05
5	10.53	369.97	387.98, 393.04, 408.99, 424.96
6	19.58	312.17	313.09, 351.00, 367.19
7	20.08	354.16	355.08, 372.19, 393.02, 409.18
8	20.45	398.10	399.13, 416.19, 437.08, 453.10
9	22.36	312.17	313.05, 367.19

***Dolichandrone serrulata* (DC.) Seem**

Family : Bignoniaceae

Synonyms *Stereospermum serrulata* DC.

Description

Deciduous tree to 25 m with narrow cylindrical crown and slender branches. Bark is pale brown, smooth or slightly flaking. Leaf is to 43 cm, once-pinnate, 3-5 pairs of leaflets, 5-14 × 3-6 cm, elliptic with tapering tip and strongly asymmetric base, usually with scattered teeth. Flower is 12-21 cm, pure white, opening at night, in short unbranched clusters of 3-7 flowers at end of twigs, 2-3 cm. Fruit is up to 85×1.8 cm, pointed, spirally twisted. Seed 2.2-2.8 × 0.5-0.8 cm, rectangular, thin with transparent wing [187].

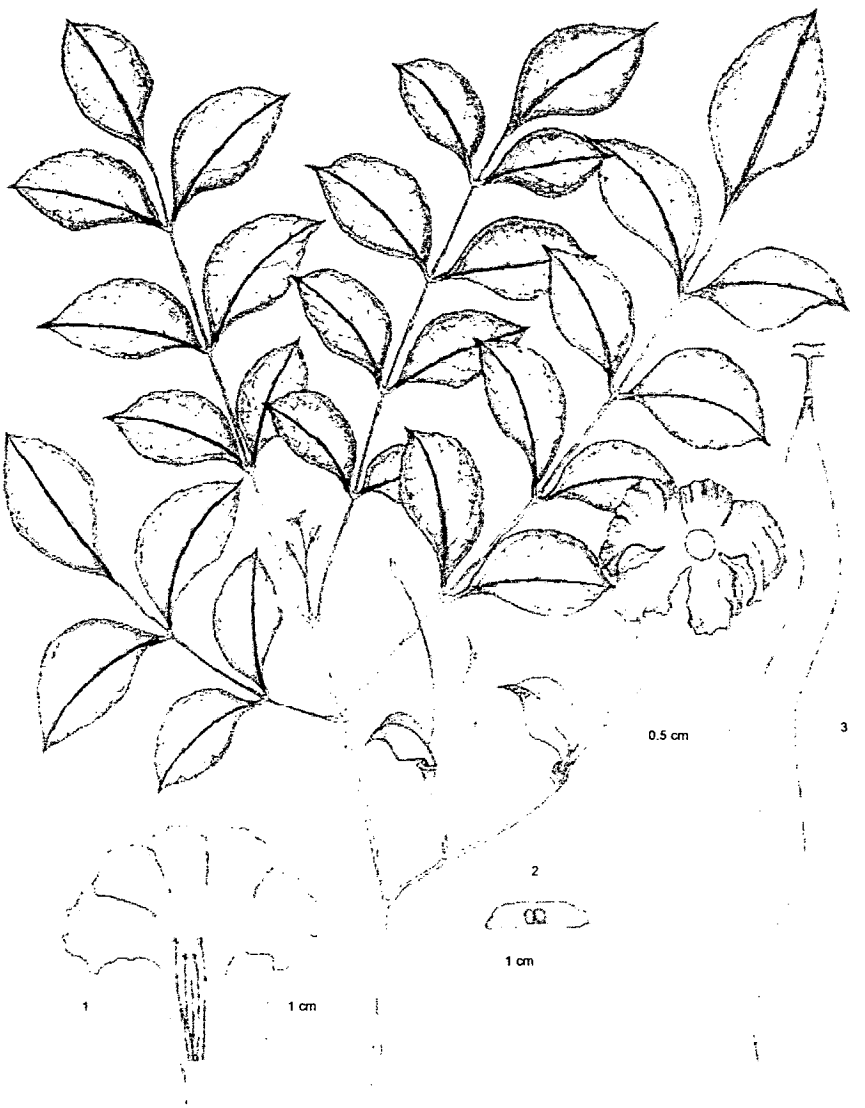


Figure 28 The flowering branch of *D. serrulata*

1. Longitudinal section of flower 2. Seed 3. Fruit

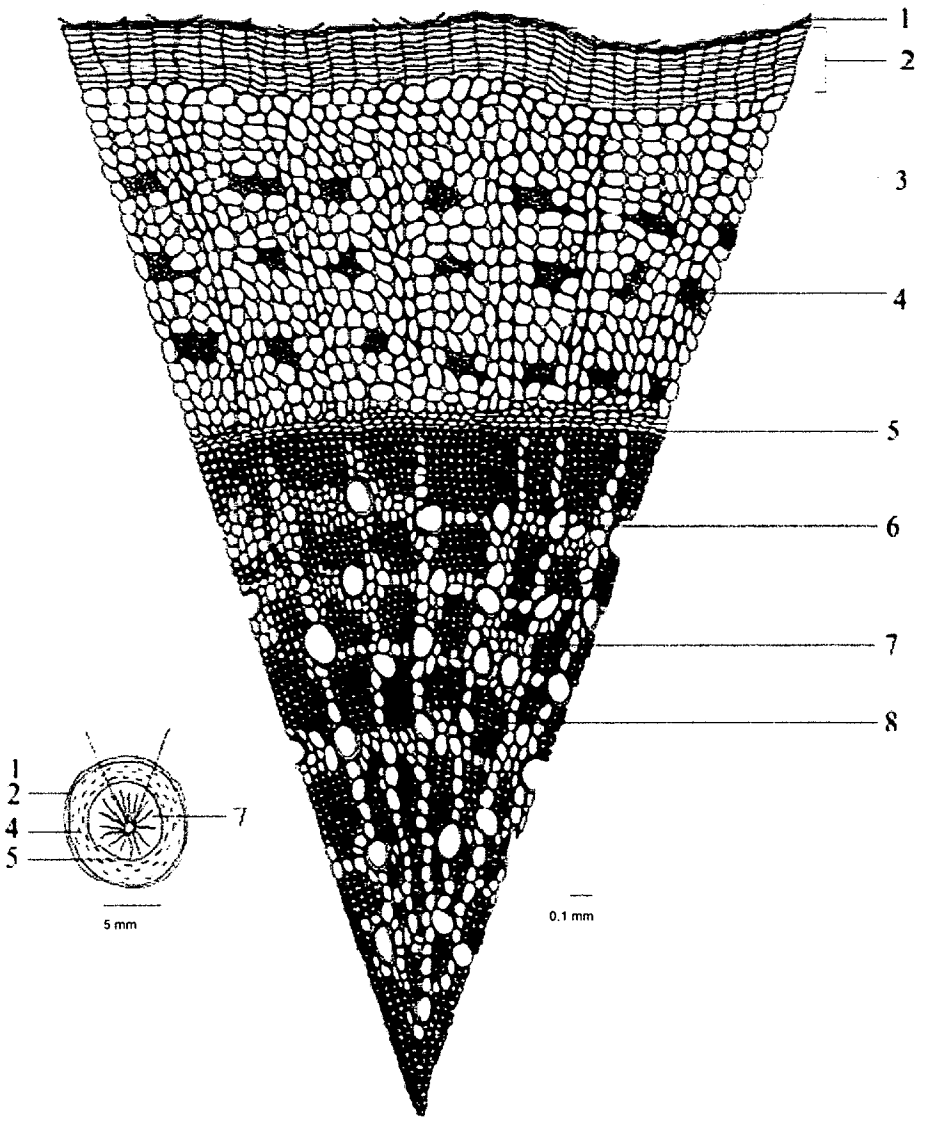


Figure 29 Transverse section of *D. serrulata* root ; 1. Epidermis 2. Periderm 3. Cortical parenchyma 4. Group of cortical fiber 5. Endodermis 6. Xylem vessel 7. Xylem ray 8. Xylem fiber

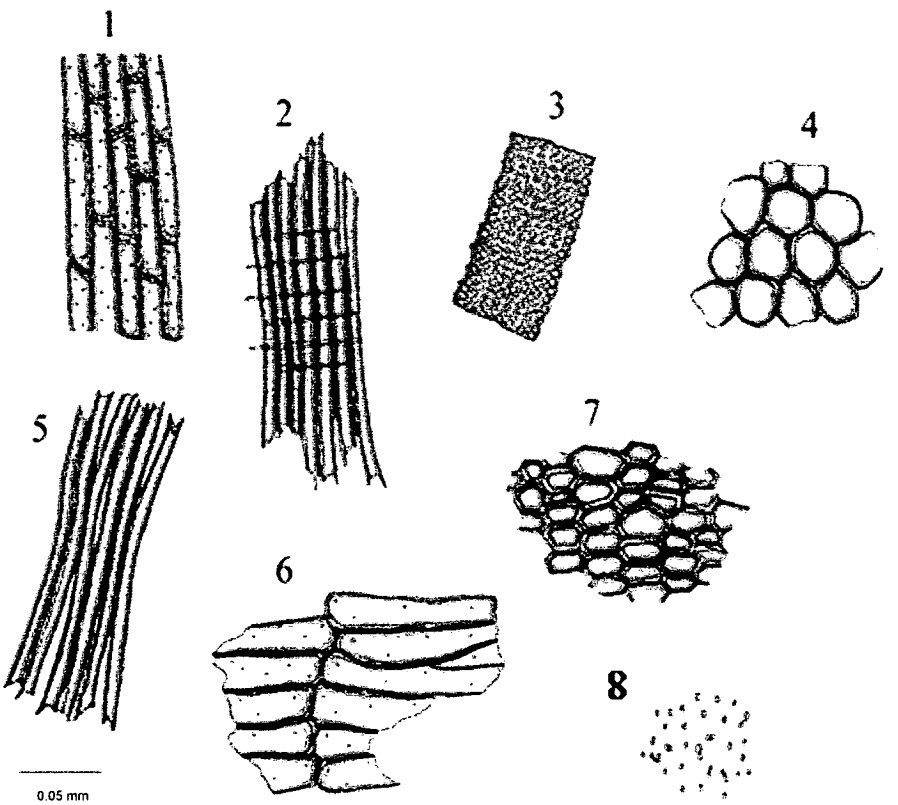


Figure 30 Powder of *D. serrulata* root; 1. Xylem parenchyma in longitudinal view 2. Xylem in radial longitudinal view 3. Fragment of pitted vessel 4. Parenchyma in transverse view 5. Fragment of fiber 6. Parenchyma in longitudinal view 7. Epidermis in surface view 8. Strach grain

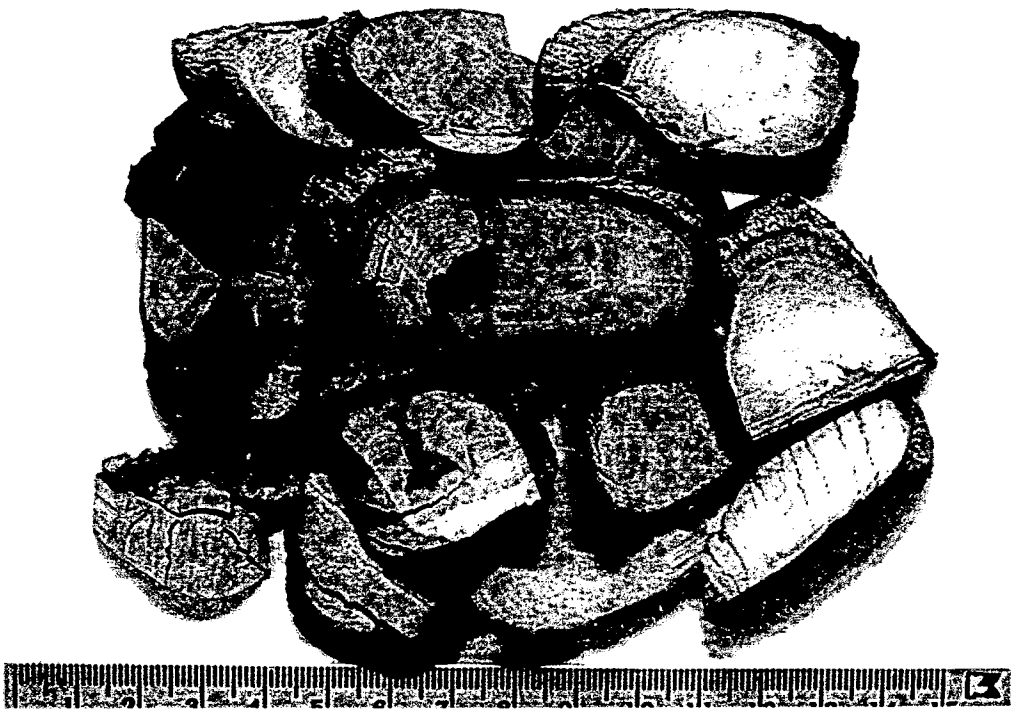


Figure 31 The root of *D. serrulata*

Table 12 Physico-chemical specification (% by weight) of *D. serrulata* root

Content (% by weight)	**Mean ±SD**	**Min-Max**	**n**
Loss on drying	7.84 ± 0.75	6.69-9.31	14
Total ash content	3.63 ± 1.06	1.12-5.30	14
Acid-insoluble ash content	0.77 ± 0.69	0.37-3.62	14
Water content	12.46 ± 1.68	10.19-14.99	14
Ethanol extractive values	4.49 ± 1.70	3.64-19.67	14
Water extractive values	10.17 ± 4.28	1.12-7.50	14

Grand mean values were calculated from 14 sources throughout Thailand. Each source was performed in triplicate.

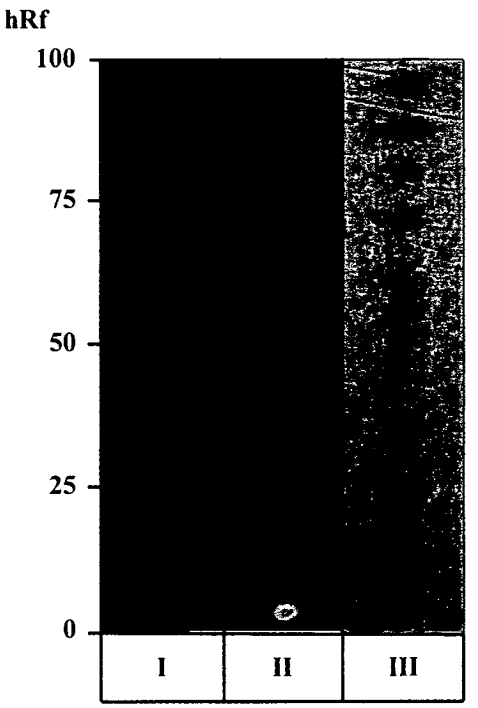


Figure 32 Thin-layer chromatogram of the methanolic extract from *D. serrulata* root

Solvent system

Chloroform : Methanol 9 : 1

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*,**

*10% sulfuric acid reagent

Preparation: conc. Sulfuric acid 10 ml. in methanol 90 ml.

**Spot color Development

Heat the plate at 105 ° C for 10 minutes after sprayed.

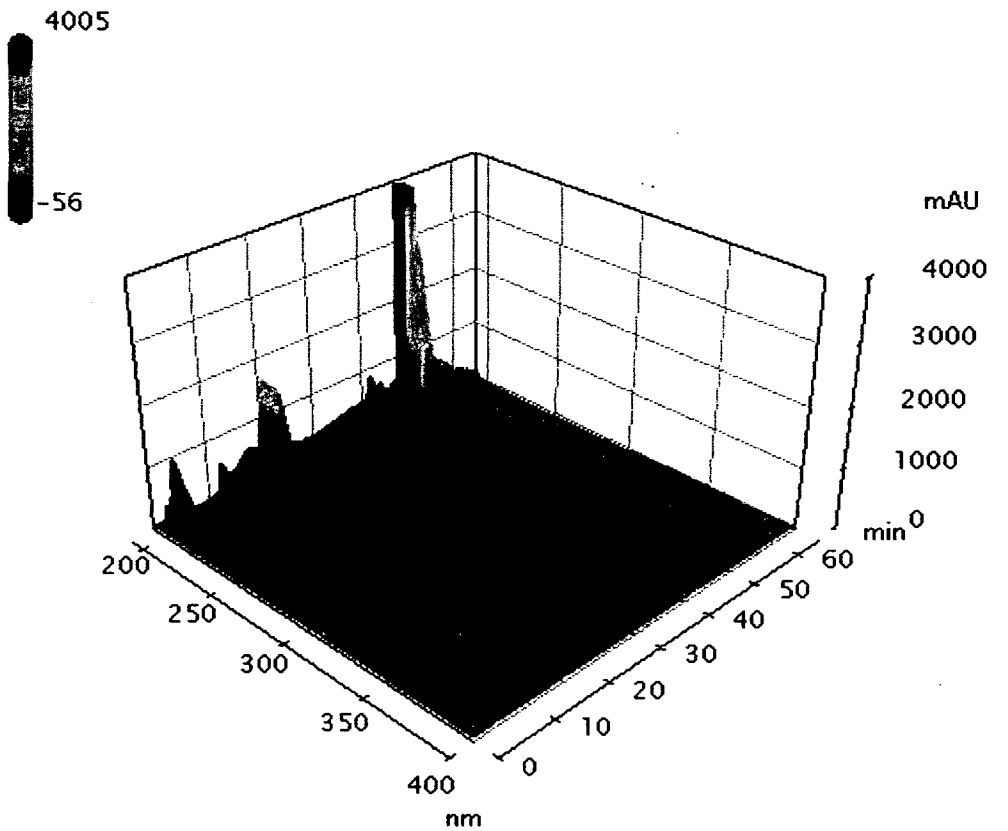


Figure 33: The 3D-HPLC profiles of ethanolic extract from *D.serrulata* root

Analysis condition

Column: Inersil ® ODS-3, C-18 column (particle size of the packing 5µm, 4.6 x 250mm)

Mobile phase: 10 mM Phosphoric acid-Acetonitrile

Linear gradient: (95:5, 65 min)

Flow rate: 0.8 mL/min

Injection volume: 10 µl

Temperature: 40°C

Wavelength: 190-400 nm.

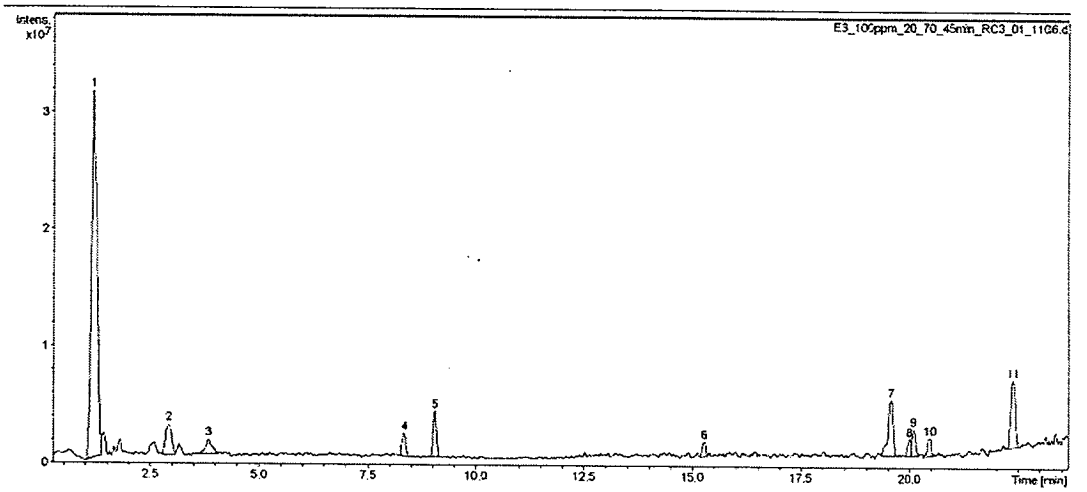


Figure 34 A representative LC/MS chromatogram from *D. serrulata* root of compound 1-11

Analysis condition

Column: Dionex C16 Acclaim RSLC PolarAdvantage column (2.1 x 100 mm, 2.2 µm,120 °A)

Mobile phase: (A) Water and (B) Acetonitrile

Linear gradient: 0-2 min (80:20%), 2-32 min (30-70%)

Flow rate: 200 µL/min

Injection volume: 5 µL of 100 ppm

Temperature: 35 °C

MS mode: UltraScan mode between m/z 70-1,500, using positive ionization

Table 13 LC- ESI-MS data and identification of constituents from the root of *D. serrulata*

Peak no.	*t*R (min)	MW	Product ions (m/z)
1	1.18	309.99	311.19, 328.15, 332.99, 349.07, 365.00
2	2.92	490.11	491.26, 508.15, 528.93, 545.13
3	3.84	646.12	647.13, 664.06, 669.11, 685.11, 701.04
4	8.33	620.16	659.26, 675.17
5	9.04	560.11	561.11, 578.08, 583.12, 599.09
6	15.26	472.27	495.45, 511.21, 527.28
7	19.56	312.16	313.13, 367.18
8	19.99	456.27	457.38, 474.32, 474.53, 495.21, 511.27
9	20.09	386.19	387.05, 404.20, 409.18, 425.16, 441.28
10	20.46	398.11	416.05, 421.00, 437.12, 453.09
11	22.37	312.17	330.34, 367.18

***Oroxylum indicum* (L.) Kurz**

Family : Bignoniaceae

Synonyms

Bignonia indica L. var. 'ALFA'a (1753), *Bignonia pentandra* Lour. (1790), *Calosanthes indica* (L.) Blume (1826).

Vernacular names

Midnight horror (En). Indonesia: pongporang (Sundanese), kayu lanang, mungli (Javanese). Malaysia: beka, bonglai, kulai. Philippines: pingka-pingkahan (Tagalog), abong-abong (Bisaya), Kamkampilan (Iloko). Cambodia: pi ka. Laos: lin may, ung ka. Thailand: phe kaa (central), litmai (northern), lin faa (north-eastern).

Distribution

Oroxylum indicum is found from India eastward to southern China and the Philippines, and throughout South-East Asia; in Indonesia eastward to Sulawesi and the Lesser Sunda Islands. Locally cultivated near human settlements.

Description

A semi-deciduous, sparingly branched tree up to 27 m tall; trunk up to 40 cm in diameter, bark grey, with prominent leaf scars, twigs thick, pithy, later hollow, lenticellate. Leaves crowded, imparipinnate, 3-4 times pinnate, 0.5-2 m long; petiole long, rachis swollen at points of insertion; stipules absent; leaflets ovate to oblong, 4-11 (-15) cm x 3-9 cm, base cuneate or mostly oblique, apex acuminate, entire, with scattered glands on the lower surface. Inflorescence an erect raceme, terminal, 25-150 cm long, peduncle and rachis partitioned. Flowers bisexual, pedicel 2-4 cm long, bracteolate; calyx coriaceous, campanulate, containing water in bud, 2-4 cm long, 1.5-2 cm in diameter, brown or dirty violet, becoming almost woody in fruit; corolla funnel-shaped, about 10 cm long, lobes 5, subequal, margin wrinkled, reddish outside, yellowish to pinkish inside; stamens 5, inserted in the throat, hairy at the base; ovary superior, 2-celled, many-ovuled. Fruit a pendent capsule, sword-shaped, 45-120 cm x 6-10 cm, valves flat, almost woody, finally black, Seed 5-9 cm x 2.5-4 cm, including the membranous and transparent wing. Seedling with epigeal germination; 96ypocotyls elongated; cotyledons leafy [188].

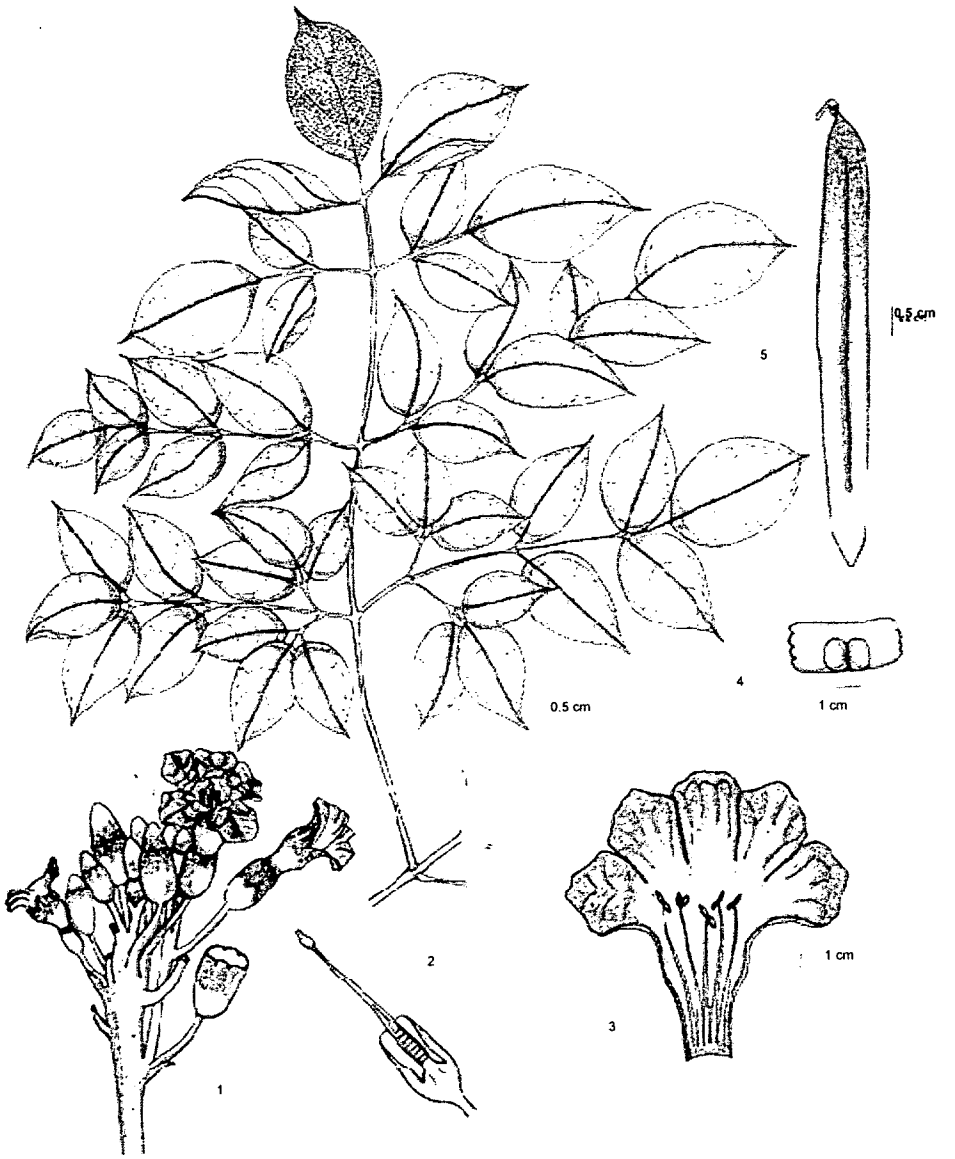


Figure 35 The flowering branch of *O. indicum*

1. Branches of flowers 2. Stamen 3. Longitudinal section of flower 4. Seed 5. Fruit

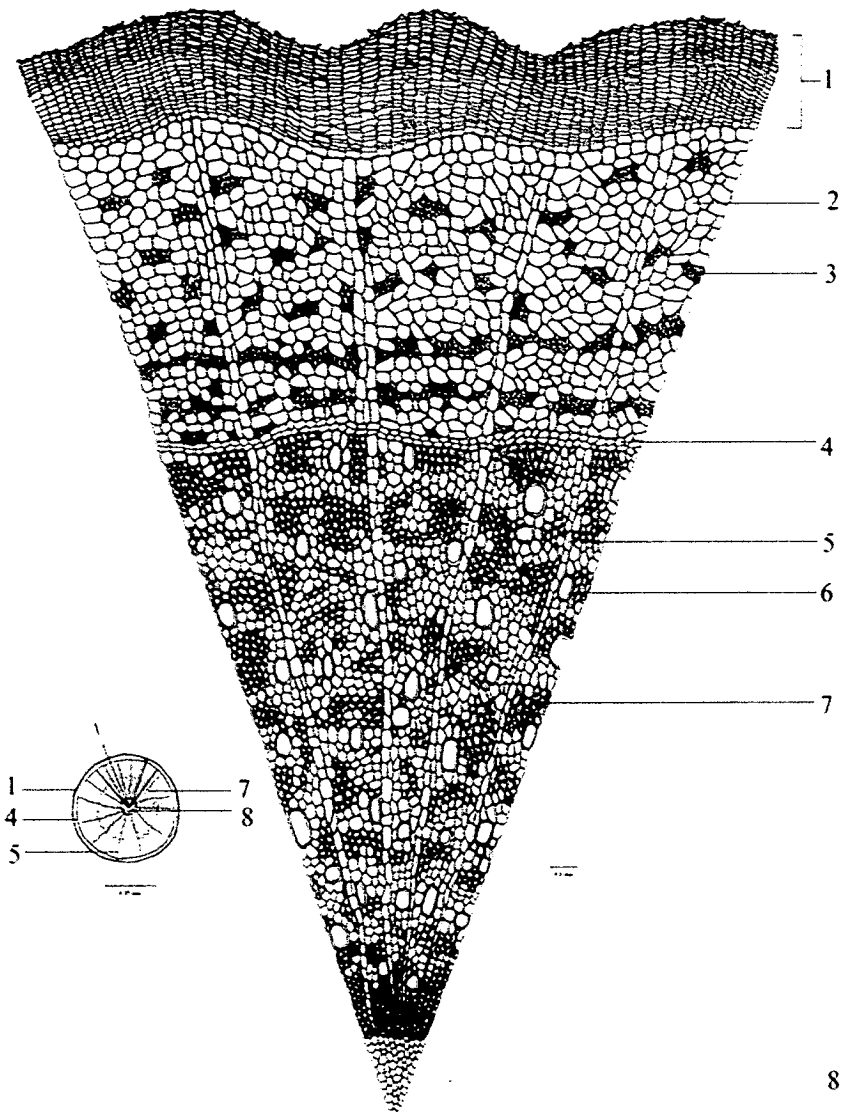


Figure 36 Transverse section of *O. indicum* root ; 1. Periderm 2. Cortical parenchyma 3. Group of cortical fiber 4. Endodermis 5. Xylem ray 6. Xylem vessel 7. Xylem fiber 8. Pith

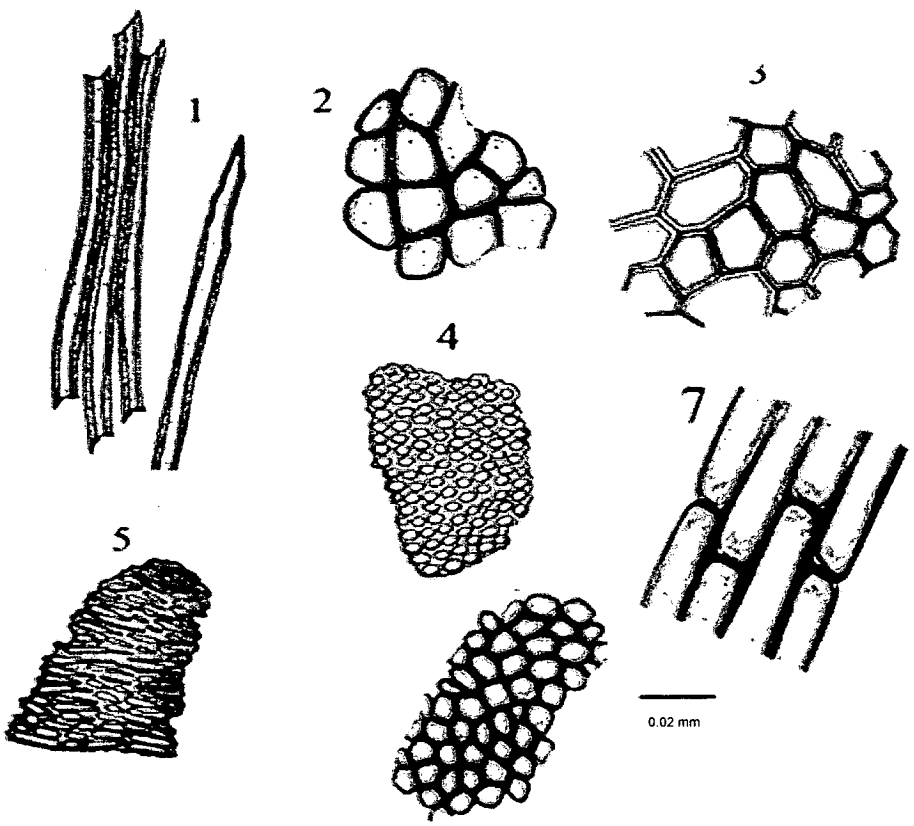


Figure 37 Powder of *O. indicum* root ; 1. Fragment of fiber 2. Epidermis in surface view 3. Cork in surface view 4. Fragment of pitted vessel 5. Fragment of reticulated vessel 6. Parenchyma in transverse view 7. Parenchyma in longitudinal view

Figure 38 The root of *O. indicum*

Table 14 Physico-chemical specification (% by weight) of *O. indicum* root

Content (% by weight)	**Mean ±SD**	**Min-Max**	**n**
Loss on drying	6.95 ± 0.92	5.50-8.14	13
Total ash content	5.59 ± 1.89	2.09-8.45	13
Acid-insoluble ash content	1.29 ± 0.76	0.38-2.60	13
Water content	10.61 ± 1.29	8.55-11.99	13
Ethanol extractive values	7.93 ± 3.26	7.01-29.18	13
Water extractive values	18.50 ± 7.69	3.48-13.88	13

Grand mean values were calculated from 13 sources throughout Thailand. Each source was performed in triplicate.

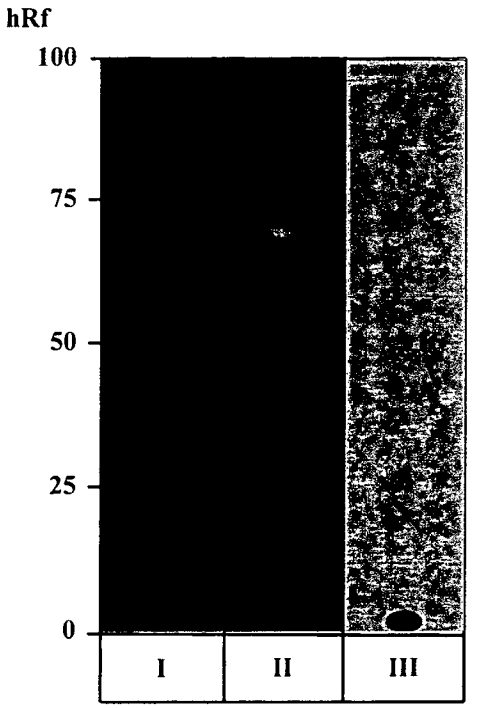


Figure 39 Thin-layer chromatogram of the methanolic extract from *O. indicum* root

Solvent system

Toluene : Ethyl acetate 3:1

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*, **

*10% sulfuric acid reagent

Preparation: conc. Sulfuric acid 10 ml in methanol 90 ml.

**Spot color Development

Heat the plate at 105 ° C for 10 minutes after sprayed.

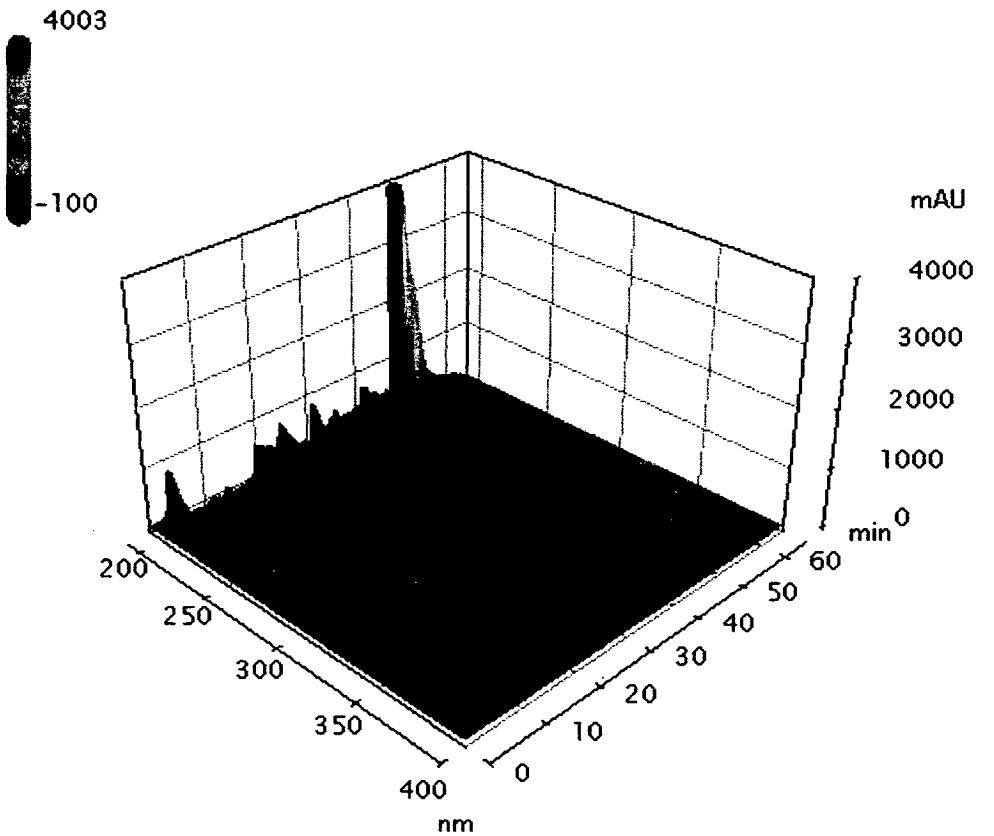


Figure 40 The 3D-HPLC profiles of ethanolic extract from *O. indicum* root

Analysis condition

Column: Inersil ® ODS-3, C-18 column (particle size of the packing 5μm, 4.6 x 250mm)

Mobile phase: 10 mM Phosphoric acid-Acetonitrile

Linear gradient: (95:5, 65 min)

Flow rate: 0.8 mL/min

Injection volume: 10 μl

Temperature: 40°C

Wavelength: 190-400 nm.

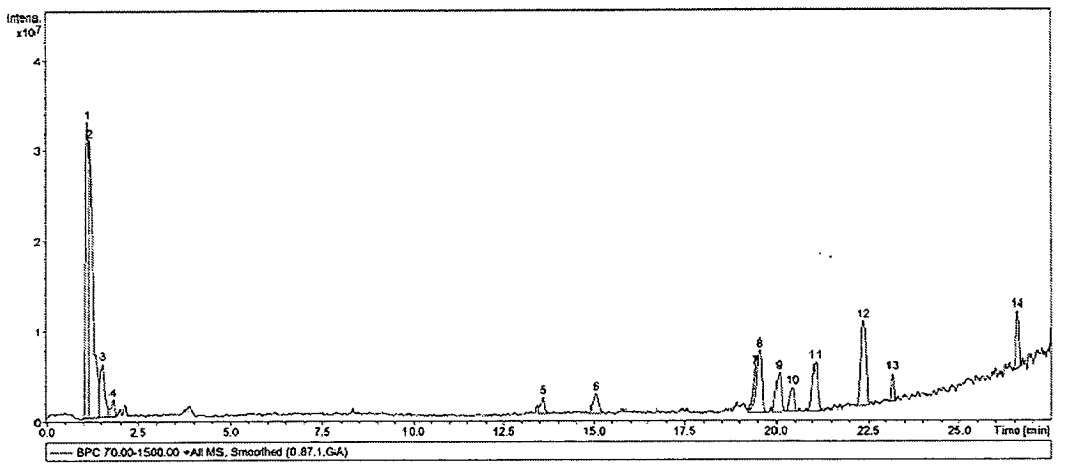


Figure 41 A representative LC/MS chromatogram from *O. indicum* root of compound 1-14

Analysis condition

Column: Dionex C16 Acclaim RSLC PolarAdvantage column (2.1 x 100 mm, 2.2 μm,120 °A)

Mobile phase: (A) Water and (B) Acetonitrile

Linear gradient: 0-2 min (80:20%), 2-32 min (30-70%)

Flow rate: 200 μL/min

Injection volume: 5 μL of 100 ppm

Temperature: 35 °C

MS mode: UltraScan mode between m/z 70-1,500, using positive ionization

Table 15 LC- ESI-MS data and identification of constituents from the root of *O. indicum*

Peak no.	*t*R (min)	MW	Product ions (m/z)
1	1.12	427.07	427.97, 472.97, 490.06, 527.09
2	1.19	427.09	473.04, 490.09, 527.12
3	1.56	176.05	177.16, 194.04, 199.06, 231.06
4	1.82	234.06	256.88, 273.03, 288.94
5	13.6	316.09	333.96, 339.07, 355.09, 371.04
6	15.04	488.07	489.06, 511.06, 527.05, 542.88
7	19.42	312.15	313.34, 335.14, 367.17
8	19.54	312.17	335.02, 351.00, 367.19
9	20.07	386.20	387.19, 404.38, 409.19, 425.16, 441.26
10	20.43	398.10	415.96, 437.10, 453.09
11	21.05	328.08	329.27, 351.19, 383.09
12	22.34	312.16	367.18
13	23.14	539.37	540.38, 557.47, 562.35, 578.35, 594.25
14	26.56	328.12	346.06, 367.00, 383.13

***Walsura trichostemon* Miq.**

Family : Meliaceae

Distribution

Walsura trichostemon Miq is a plant of family Meliaceae that has been found in evergreen forest drought throughout Southeast Asia such as Myanmar, Cambodia. Thailand found in North, Northeast and southeastern, which know the local name of Musk Mallow tree [189].

Description

Botany evergreen of briefly deciduous trees, very rarely with latex of sap. Leaves odd-pinnate, stalks swollen and jointed. Alternate, spirally arranged, leaflets usually opposite, no stipules. Flowers mostly white or yellow, regular, bisexual, in branched clusters at upper leaf axils, 4-5 free spreading petals, stamens longer than petals, style short, disc ring-like. Fruits fleshy or leathery, not splitting, 1-2 seeds with aril.

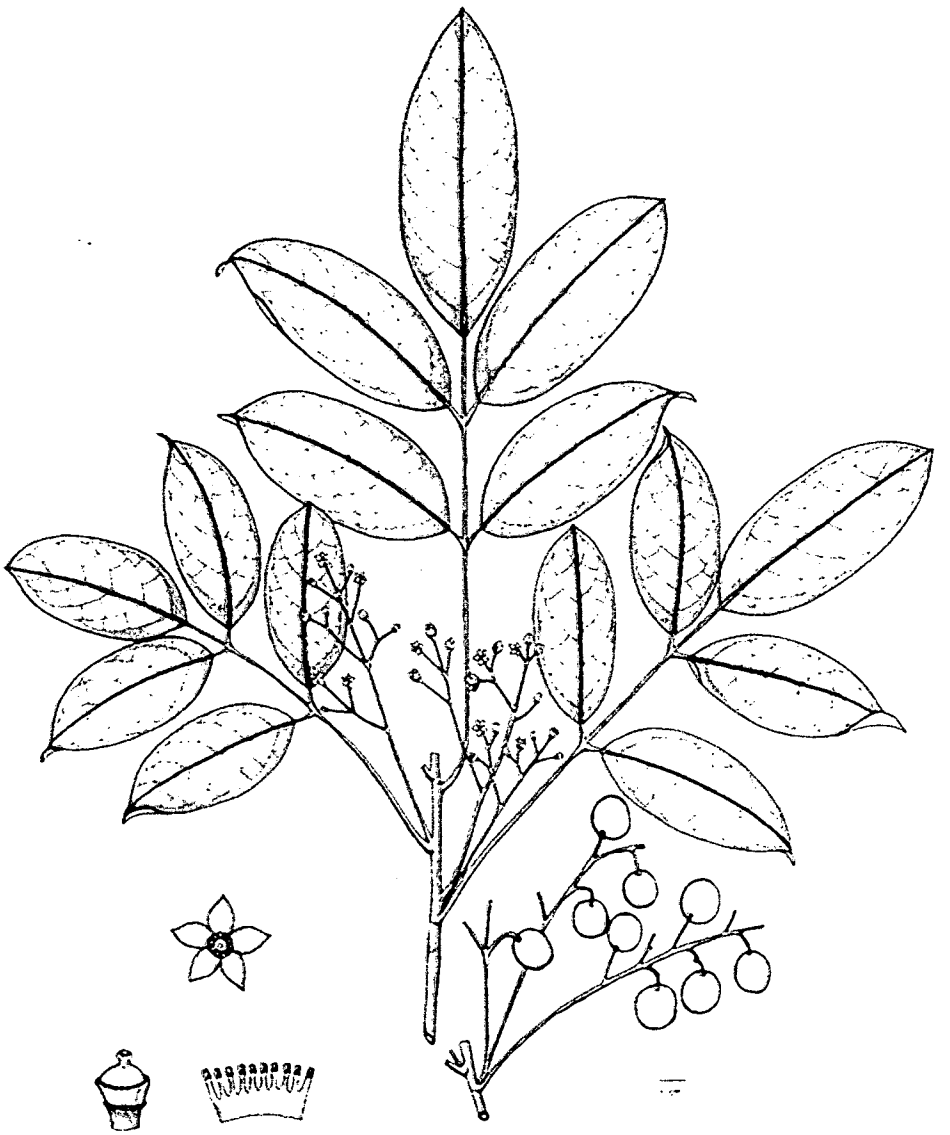


Figure 42 The fruiting branch of *W. trichostemon*

1. Branches of fruits 2. Flower 3. Stamens 4. Pistil

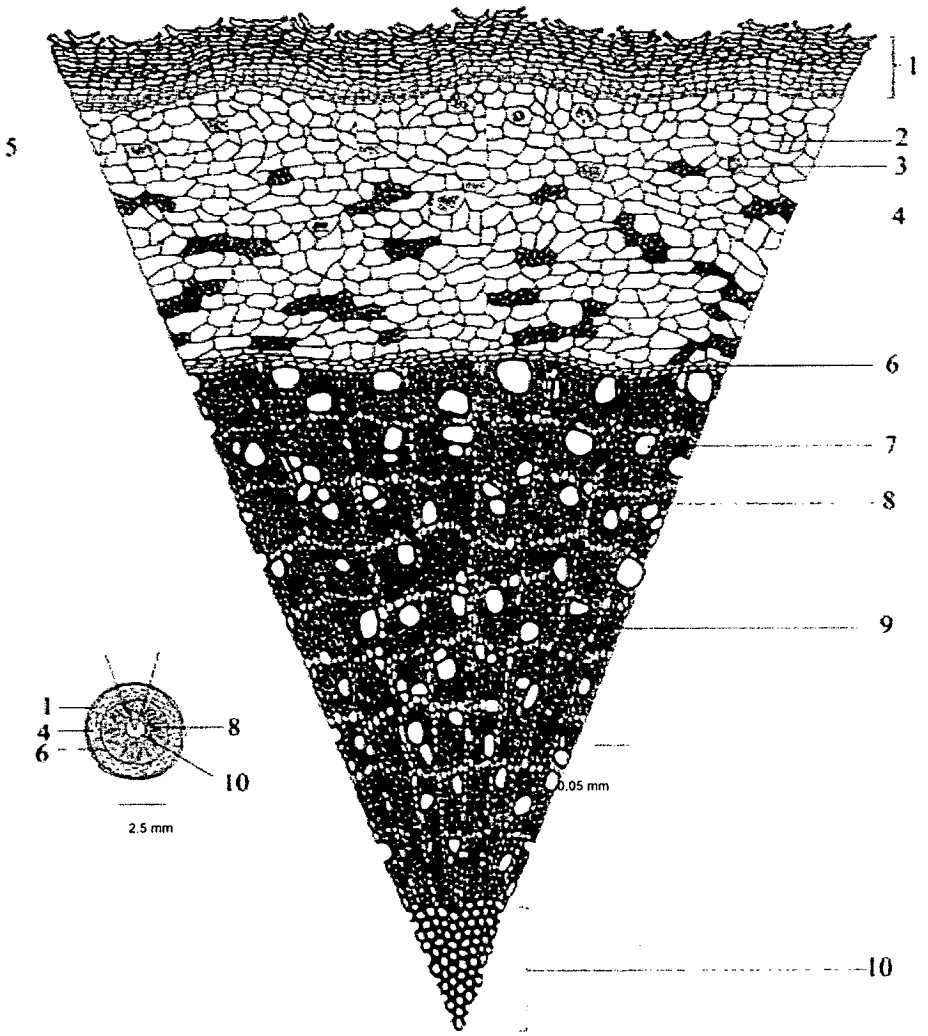


Figure 43 Transverse section of *W. trichostemon* root; 1. Periderm 2. Cortical parenchyma 3. Prismatic crystals in reserved parenchyma 4. Cortical fiber 5. Strach granule in reserved parenchyma 6. Endodermis 7. Xylem vessel 8. Xylem ray 9. Xylem fiber 10. Pith

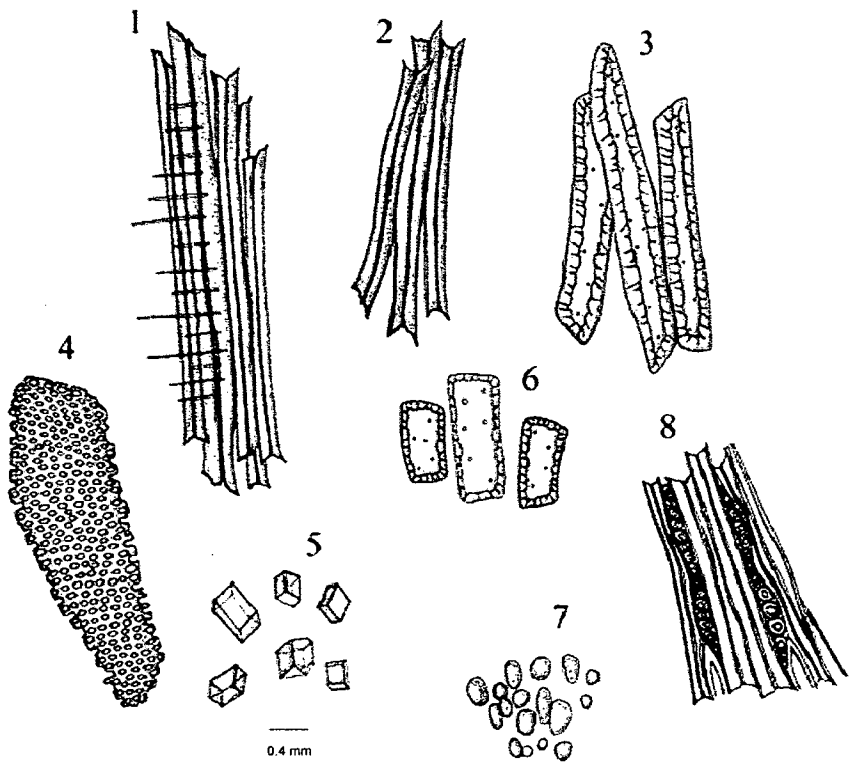


Figure 44 Powder of *W. trichostemon* root ; 1. Xylem in radial longitudinal view 2. Fragment of fiber 3. Sclereid in longitudinal view 4. Fragment of pitted vessel 5. Prism crystal of calcium oxalate 6. Sclereid in transverse view 7. Starch grain 8. Xylem in tangential longitudinal view

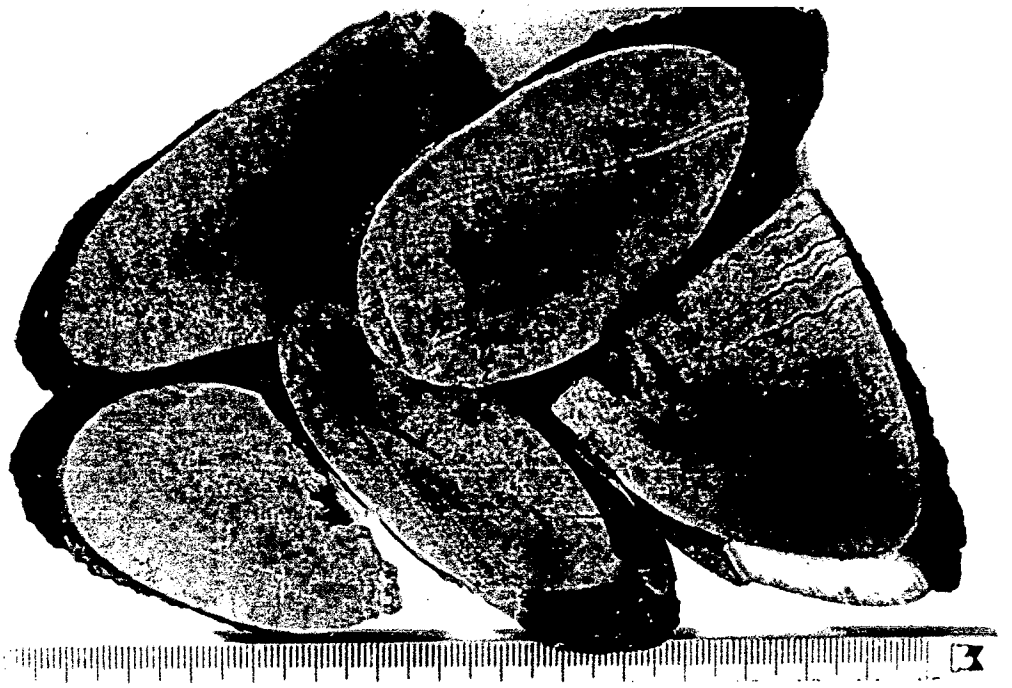


Figure 45 The root of *W. trichostemon*

Table 16 Physico-chemical specification (% by weight) of *W. trichostemon* root

Content (% by weight)	**Mean ±SD**	**Min-Max**	**n**
Loss on drying	7.09 ± 0.82	5.79-8.23	13
Total ash content	2.82 ± 1.13	1.60-5.80	13
Acid-insoluble ash content	0.56 ± 0.24	0.31-0.96	13
Water content	13.20 ± 1.56	11.74-16.01	13
Ethanol extractive values	6.37 ± 3.32	3.57-9.88	13
Water extractive values	5.98 ± 2.21	1.64-7.50	13

Grand mean values were calculated from 13 sources throughout Thailand. Each source was performed in triplicate.

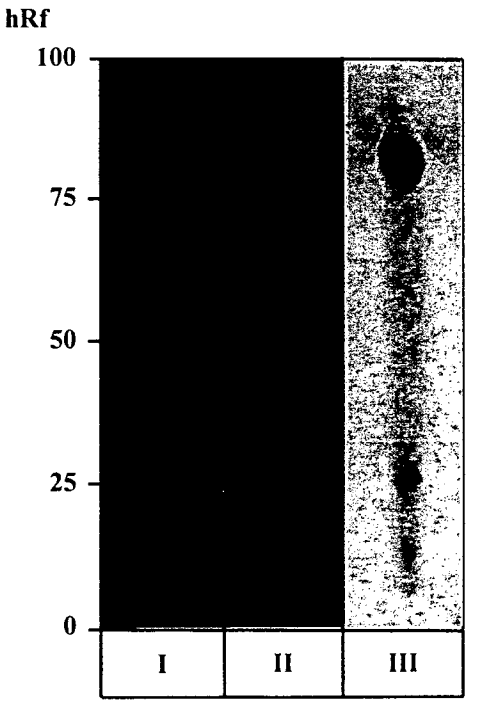


Figure 46 Thin-layer chromatogram of the methanolic extract from *W. trichostemon* root

Solvent system

n-butanol : acetic acid : water 4 : 1 : 5

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*,**

*10% sulfuric acid reagent

Preparation: conc. Sulfuric acid 10 ml. in methanol 90 ml.

**Spot color Development

Heat the plate at 105 ^{o} C for 10 minutes after sprayed.

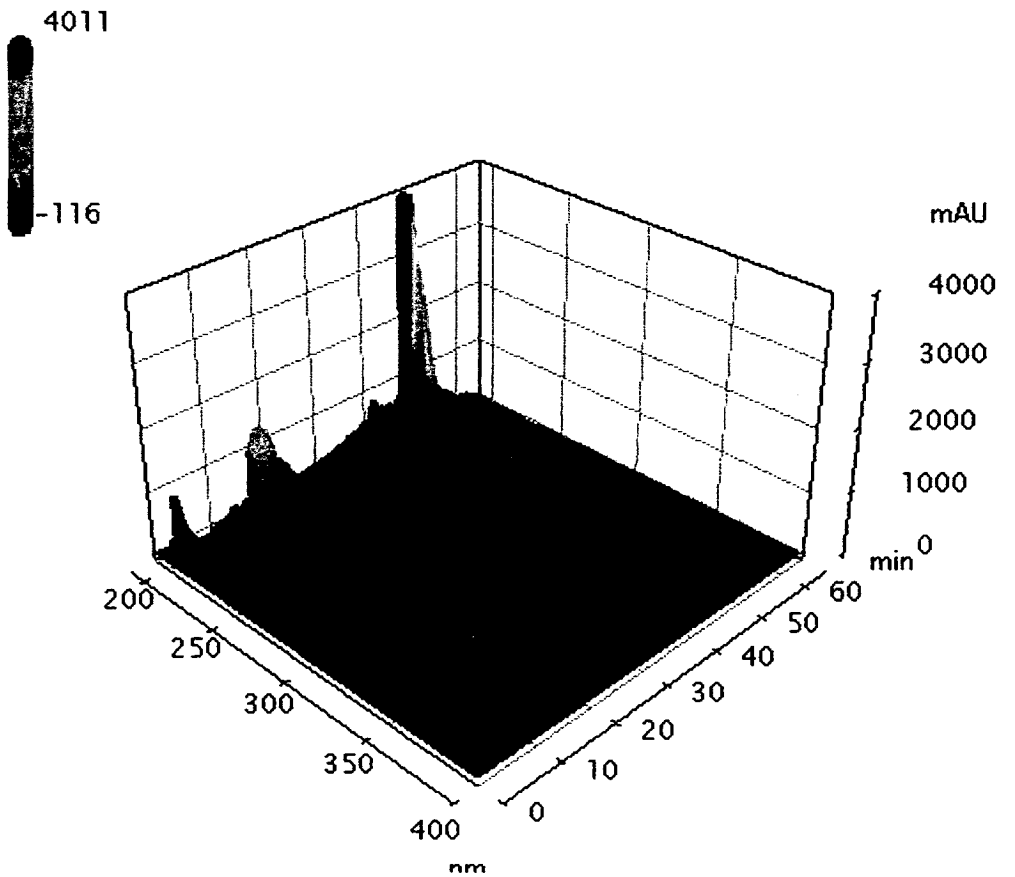


Figure 47 The 3D-HPLC profiles of ethanolic extract from *W.trichostemon* root

Analysis condition

Column: Inersil ® ODS-3, C-18 column (particle size of the packing 5µm, 4.6 x 250mm)

Mobile phase: 10 mM Phosphoric acid-Acetonitrile

Linear gradient: (95:5, 65 min)

Flow rate: 0.8 mL/min

Injection volume: 10 µl

Temperature: 40°C

Wavelength: 190-400 nm.

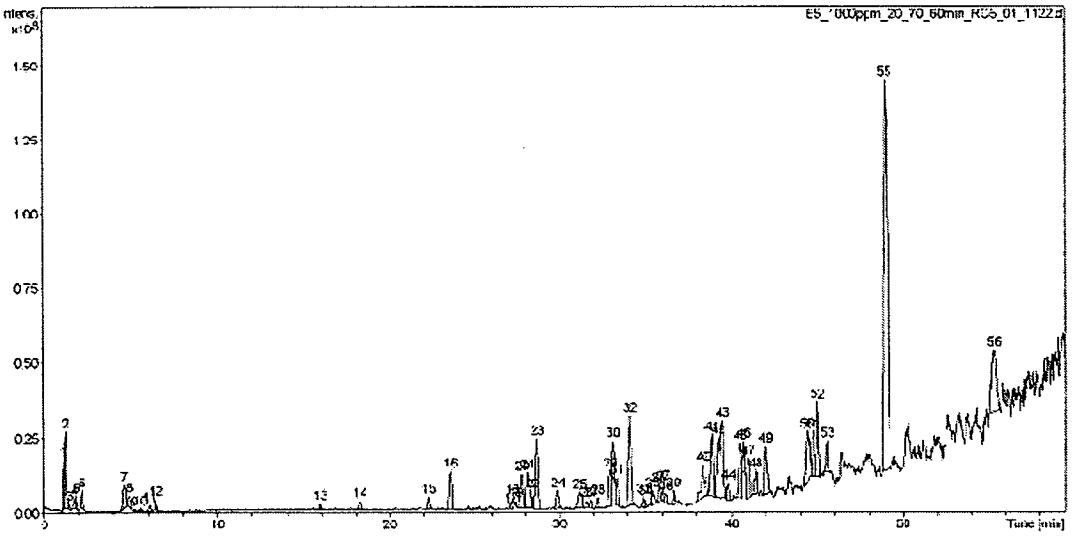


Figure 48 A representative LC/MS chromatogram from *W. trichostemon* root of compound 1-56

Analysis condition

Colum: Dionex C16 Acclaim RSLC PolarAdvantage column (2.1 x 100 mm, 2.2 μm,120 °A)

Mobile phase: (A) Water and (B) Acetonitrile

Linear gradient: 0-2 min (85:15%), 2-60 min (30-70%)

Flow rate: 200 μL/min

Injection volume: 2 μL of 1000 ppm

Temperature: 35 °C

MS mode: UltraScan mode between m/z 70-1,500, using positive ionization

Table 16 LC- ESI-MS data and identification of constituents from the root of *W. trichostemon*

Peak no.	*t*R (min)	MW	Product ions (m/z)
1	1.15	233.97	234.92, 256.79, 288.99
2	1.25	148.04	171.13, 203.05
3	1.47	344.05	344.97, 362.08, 367.05, 383.00, 398.94
4	1.72	302.02	303.01, 325.03, 340.98, 357.30
5	1.84	466.12	484.20, 489.16, 505.11, 521.08
6	2.21	278.06	279.05, 279.26, 317.05, 333.01
7	4.65	566.18	584.00, 605.19 , 621.15
8	4.9	566.20	567.07, 584.10, 605.20, 621.17
9	5.18	536.19	537.39, 559.10, 575.13, 591.24
10	5.58	356.04	357.15, 379.15, 395.06, 411.01
11	6.1	536.20	537.21, 575.18, 591.16
12	6.44	568.19	568.97, 586.00, 586.31, 607.19, 623.16
13	15.94	544.21	545.26, 562.21, 567.11, 583.20, 599.15
14	18.2	560.23	561.12, 578.14, 583.17, 599.22, 615.25
15	22.28	602.23	620.05, 641.17, 657.25
16	23.54	512.19	513.10, 551.15, 567.20
17	27.12	602.30	641.27, 657.31

Peak no.	*t*R (min)	MW	Product ions (m/z)
18	27.22	512.20	551.29, 567.21
19	27.42	558.24	581.08, 613.26
20	27.75	570.21	571.39, 588.44, 593.46, 609.36, 625.22
21	28.08	560.25	561.16, 578.01, 599.24, 615.21
22	28.37	546.31	564.31, 569.28, 585.30, 601.25
23	28.65	544.22	562.04, 567.20, 583.11, 599.24
24	29.87	632.31	633.25, 671.29, 687.25
25	31.16	558.24	559.20, 576.35, 613.26
26	31.53	468.19	486.38, 507.13, 523.20
27	31.86	554.23	555.24, 572.40, 609.24
28	32.23	670.32	671.29, 688.21, 709.31, 725.35
29	32.99	488.23	506.16, 543.24
30	33.15	670.34	671.43, 693.43, 725.36
31	22.36	496.18	551.20
32	34.14	554.21	555.28, 572.39, 593.28, 609.22
33	34.92	554.22	555.00, 572.31, 577.07, 609.24
34	35.06	586.21	604.42, 609.15, 625.13, 641.23
35	35.46	642.38	643.42, 665.22, 681.32, 697.40

Peak no.	*t*R (min)	MW	Product ions (m/z)
36	35.82	528.24	567.43, 583.25
37	36.09	644.32	645.34, 662.23, 667.13, 683.29, 699.34
38	36.29	584.34	585.20, 639.36
39	36.70	526.20	544.18, 549.19, 565.17, 581.37
40	38.39	698.38	721.50, 737.38, 753.39
41	38.84	468.21	469.07, 486.18, 491.09, 523.23
42	39.17	598.38	599.21, 637.27, 653.40
43	39.47	598.38	599.32, 616.33, 616.52, 653.40
44	39.81	656.39	659.45, 711.40
45	40.48	712.33	713.34, 730.30, 751.27, 767.35
46	40.68	712.40	735.30, 767.42
47	40.91	712.38	730.30, 751.45, 767.40
48	41.37	598.37	621.22, 621.50, 653.39
49	41.97	626.38	627.14, 644.39, 681.40
50	44.39	696.41	719.55, 735.46, 751.43
51	44.73	640.40	641.45, 663.58, 695.42
52	45.00	640.40	641.42, 663.58, 679.31, 695.42
53	45.55	652.33	670.19, 675.33, 707.34

Peak no.	*t*R (min)	MW	Product ions (m/z)
54	48.95	452.21	507.22
55	48.95	452.21	507.22
56	55.35	447.33	448.36, 470.31, 470.51, 502.34

Ben-Cha-Moon-Yai remedy

HPLC chromatogram and Mass spectrometric data of BMY extract prepared by the extracts combination according to an equal weight of each root was demonstrated in figure 49-50 and table 18.

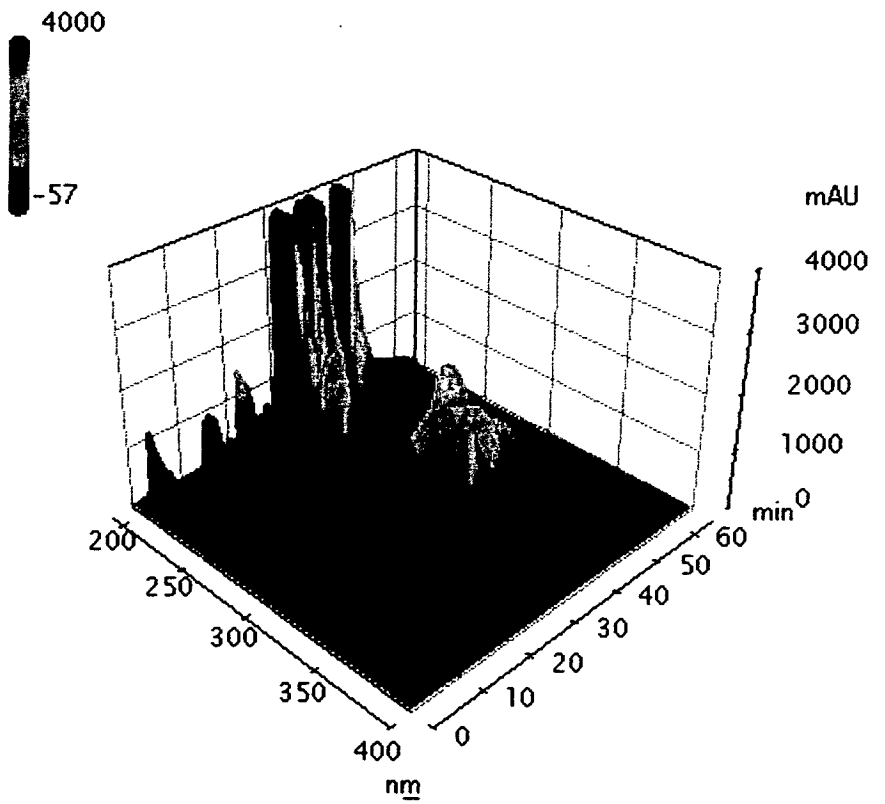


Figure 49 The 3D-HPLC profiles of ethanolic extract from Ben-Cha-Moon-Yai remedy

Analysis condition

Colum: Inersil ® ODS-3, C-18 column (particle size of the packing 5μm, 4.6 x 250mm) **Mobile phase:** 10 mM Phosphoric acid-Acetonitrile **Linear gradient:** (95:5, 65 min) **Flow rate:** 0.8 mL/min **Injection volume:** 10 μl **Temperature:** 40°C **Wavelength:** 190-400 nm.

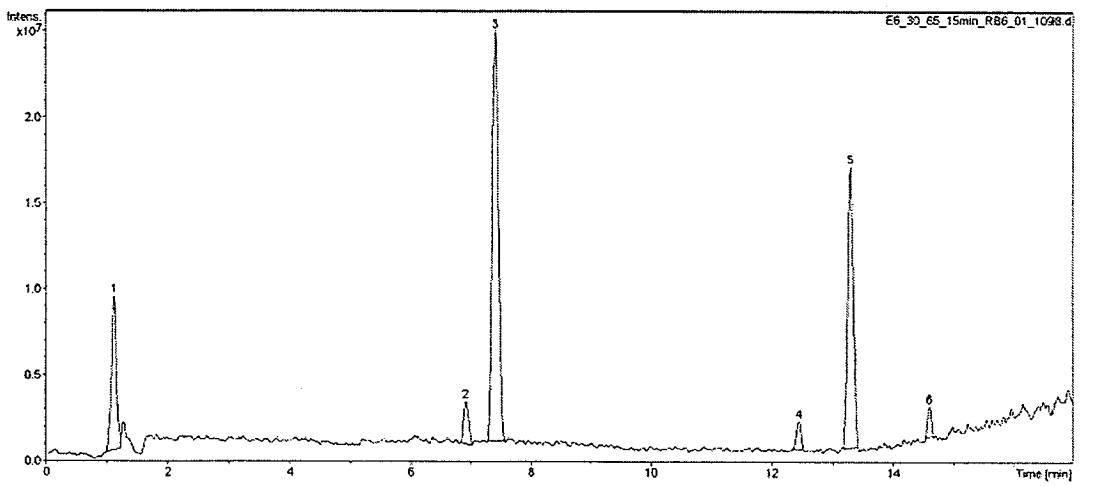


Figure 50 A representative LC/MS chromatogram from Ben-Cha-Moon-Yai remedy extract of compound 1-6

Analysis condition

Colum: Dionex C16 Acclaim RSLC PolarAdvantage column (2.1 x 100 mm, 2.2 μm,120 °A)

Mobile phase: (A) Water and (B) Acetonitrile

Linear gradient: 0-2 min (70:30%), 2-40 min (35-65%)

Flow rate: 200 μL/min

Injection volume: 5 μL of 10 ppm

Temperature: 35 °C

MS mode: UltraScan mode between m/z 70-1,500, using positive ionization

Table 18 LC- ESI-MS data and identification of constituents from the root of Ben-Cha-Moon-Yai remedy

Peak no.	*t*R (min)	MW	Product ions (m/z)
1	1.28	189.9	191.1, 213.0, 229.0, 244.9
2	6.89	259.0	260.0, 277.0, 282.0, 297.9, 314.0
3	7.54	300.1	301.1, 318.1, 323.2, 339.1, 355.1
4	12.21	298.1	299.0, 316.2, 321.0, 337.1, 353.0
5	13.35	344.1	345.1, 362.2, 367.0, 383.1, 399.1
6	14.79	298.1	299.1, 316.0, 321.0, 337.1, 353.0

Plant extraction

Table 19 showed crude extracted yields of each five root species in BMY remedy

Plant name	Yield of ethanol extract	Yield of water extract	Total yield (%)
Aegle marmelos	8.5307	5.9180	14.4487
Dolichandrone serrulata	12.4625	3.2434	15.7058
Dimocarpus longan	6.0784	3.6550	9.7334
Oroxylum indicum	16.6441	7.0639	23.7080
Walsura trichostemon	10.1250	4.6900	14.8150

Cytotoxicity assay

The results from the brine shrimp lethality testing showed in table 20. It was found that the ethanol extract of *A. marmelos* exhibited the highest toxicity against brine shrimp nauplii with LC_{50} of 53.5 µg/ml while BMY remedy showed LC_{50} of 537.3 µg/ml. The remaining extracts exhibited LC_{50} of more than 1000 µg/ml.

Table 20 Brine shrimp lethality (LC_{50}) of the extracts of Ben-Cha-Moon Yai remedy and its ingredient

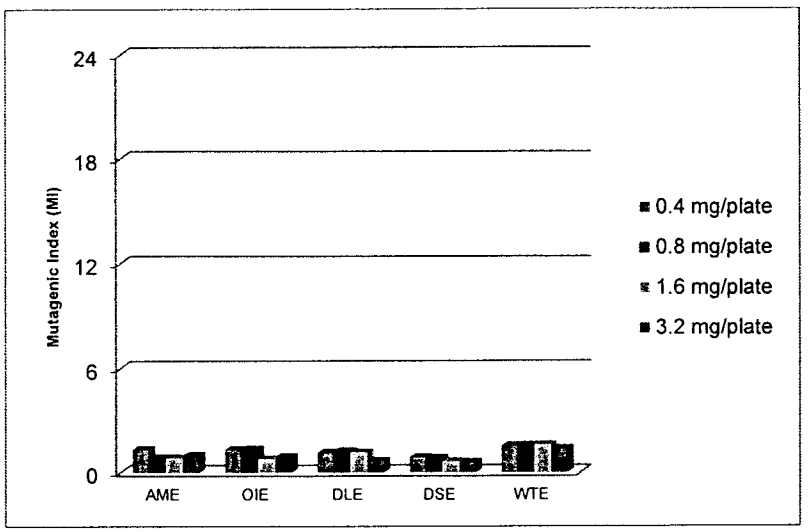
Plant name	**Brine shrimp test Mean LC_{50} (µg/ml), 24 hrs.**	
	Ethanol extract	**Water extract**
Oroxylum indicum	> 1000	> 1000
Aegle marmelos	**53.5**	> 1000
Dimocarpus longan	> 1000	> 1000
Walsura trichostemon	> 1000	> 1000
Dolichandrone serrulata	> 1000	> 1000
Ben Cha Moon Yai remedy	**537.3**	

Mutagenicity assay

MI values of BMY remedy and root extracts obtained by the Ames test were shown in figure 51-55. The result demonstrated that only the water extracts from the root of *A. marmelos* exhibited highest direct mutagenicity on both strains. The extract induced 102.33 ± 39.11 (MI=2.95) and 787.67 ± 26.84 (MI=22.72) revertant colonies at 1.6 mg/plate and at 3.2 mg/plate respectively to strain TA98 (figure 51B) and 819.5±6.36 (MI=5.17) revertant colonies at 3.2 mg/plate to strain TA100 (figure 52B).

It was observed that both ethanol and water extracts of all roots (figure 53-55) and BMY remedy exhibited their mutagenic effect after they were treated with sodium nitrite (nitrosation) under acidic condition without metabolic activation on both strains. BMY remedy extracts at all tested concentrations exhibited a positive response of mutagenicity after nitrite treated 1-aminopyrene in an acidic condition against *Salmonella typhimurium* TA98 by induced 102 ± 17.09 (MI=5.83), 191 ± 75.43 (MI=10.99), 251.67 ± 63.57 (MI=14.38) and 347 ± 18.36 (MI=19.83) revertant colonies and strain TA100 of 215 ± 35.64 (MI=3.86), 194.67 ± 24.99 (MI=4.4), 276 ± 19.52 (MI=5.56) and 392.33 ± 62.61 (MI=7.9) revertant colonies with dose-response relationship (figure 55B). However, there were no mutagenic effects exhibited by the ethanolic extract of *D. serrulata* and *D. longan* and the water extracts of *D. serrulata* to strain TA98 and the ethanolic extracts of *D. serrulata* and *W. trichostemon* towards strain TA100.

51A (TA98)



51B (TA98)

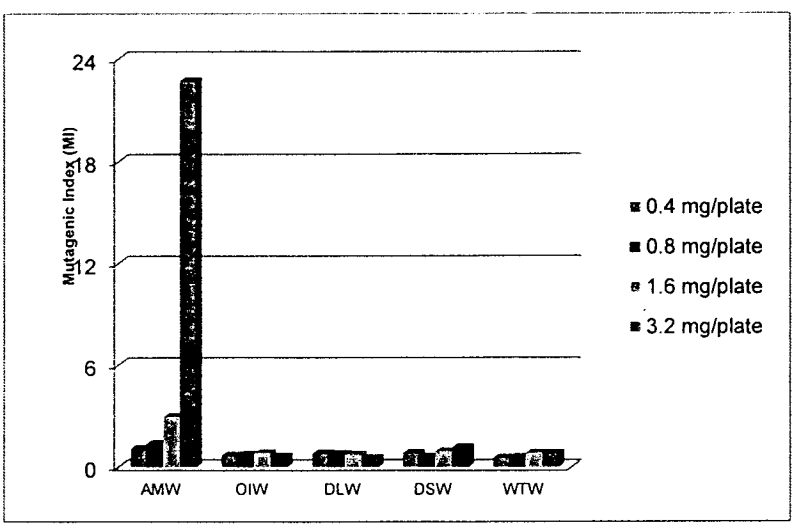
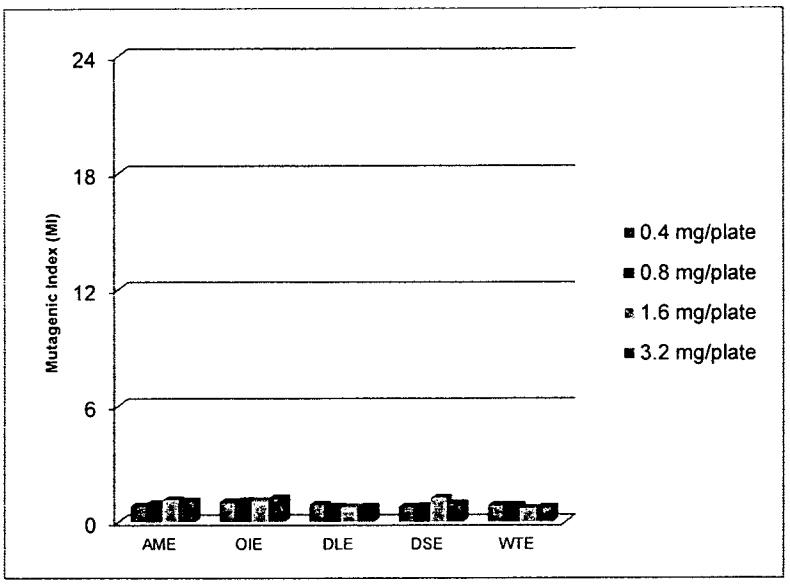


Figure 51 The mutagenic index (MI) induced by the ethanol **(51A)** and water extracts **(51B)** from each plant species of Ben-Cha-Moon-Yai remedy without nitrite treated on *Salmonella typhimurium* strains TA98 using Ames test. Abbreviations including: *Aegle marmelos*: AM, *Oroxylum indicum*: OI, *Dimocarpus longan*: DL, *Dolichandrone serrulata*: DS, *Walsura trichostemon*: WT, E: ethanol extract, W: water extract.

52A (TA100)



52B (TA100)

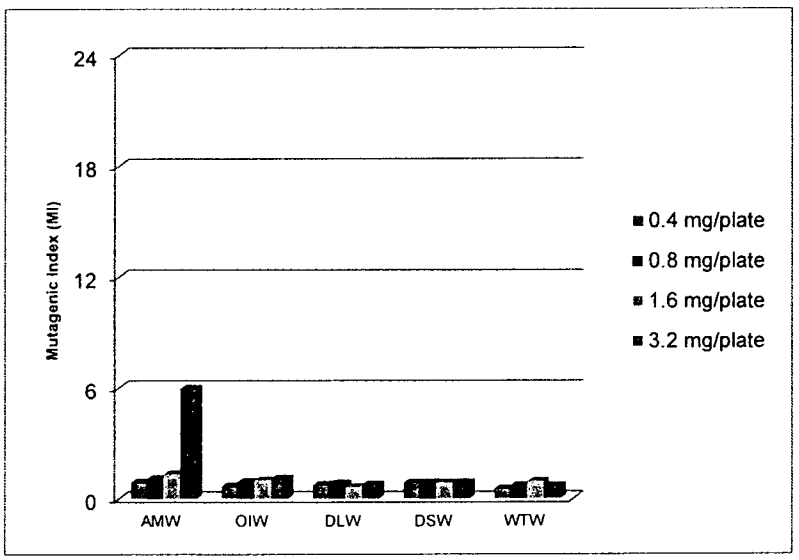
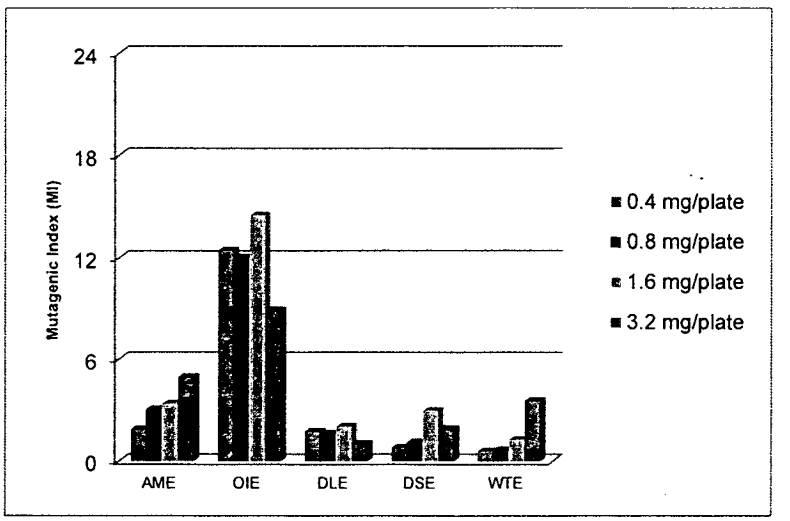


Figure 52 The mutagenic index (MI) induced by the ethanol **(2A)** and water extracts **(2B)** from each plant species of Ben-Cha-Moon-Yai remedy without nitrite treated on *Salmonella typhimurium* strains TA100 using Ames test. Abbreviations including: *Aegle marmelos*: AM, *Oroxylum indicum*: OI, *Dimocarpus longan*: DL, *Dolichandrone serrulata*: DS, *Walsura trichostemon*: WT, E: ethanol extract, W: water extract.

53A (TA98)



53B (TA98)

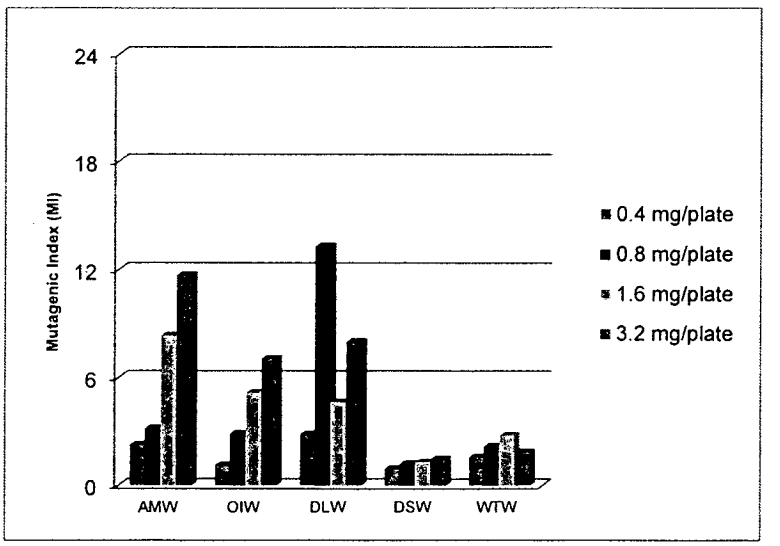
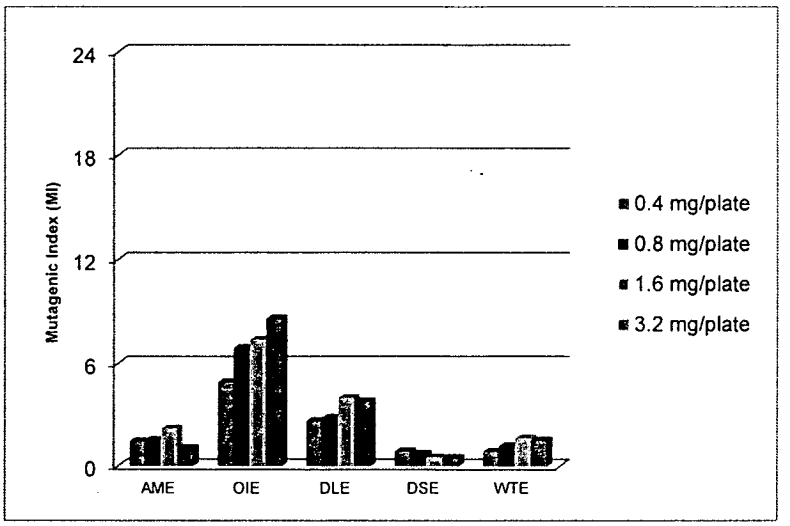


Figure 53 The mutagenic index (MI) induced by the ethanol **(3A)** and water extracts **(3B)** from each plant species of Ben-Cha-Moon-Yai remedy with nitrite treated 1-aminopyrene on *Salmonella typhimurium* strain TA98 using Ames test. Abbreviations including: *Aegle marmelos*: AM, *Oroxylum indicum*: OI, *Dimocarpus longan*: DL, *Dolichandrone serrulata*: DS, *Walsura trichostemon*: WT, E: ethanol extract, W: water extract.

54A (TA100)



54B (TA100)

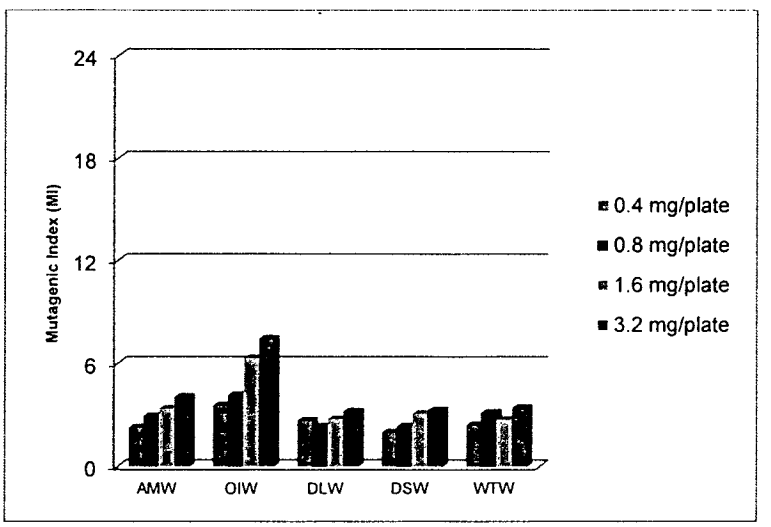
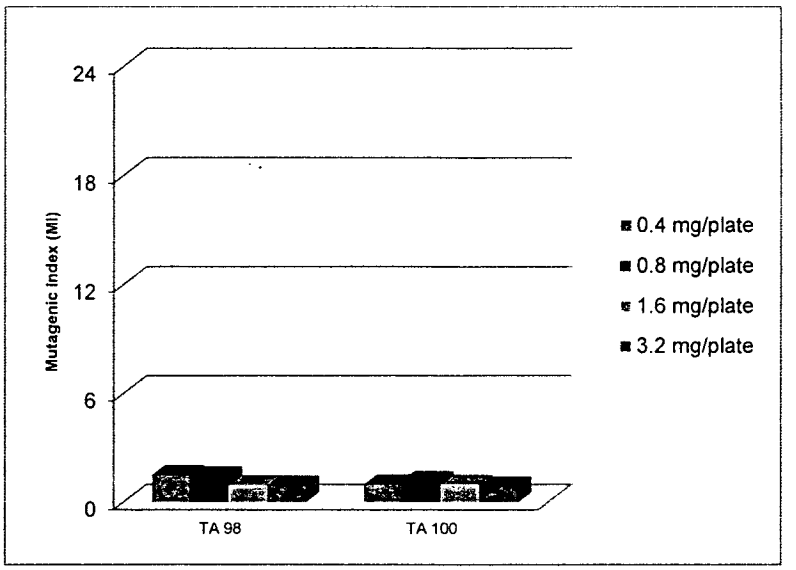


Figure 54 The mutagenic index (MI) induced by the ethanol **(4A)** and water extracts **(4B)** from each plant species of Ben-Cha-Moon-Yai remedy with nitrite treated 1-aminopyrene on *Salmonella typhimurium* strain TA100 using Ames test. Abbreviations including: *Aegle marmelos*: AM, *Oroxylum indicum*: OI, *Dimocarpus longan*: DL, *Dolichandrone serrulata*: DS, *Walsura trichostemon*: WT, E: ethanol extract, W: water extract.

55A (without nitrite treatment)



55B (with nitrite treatment)

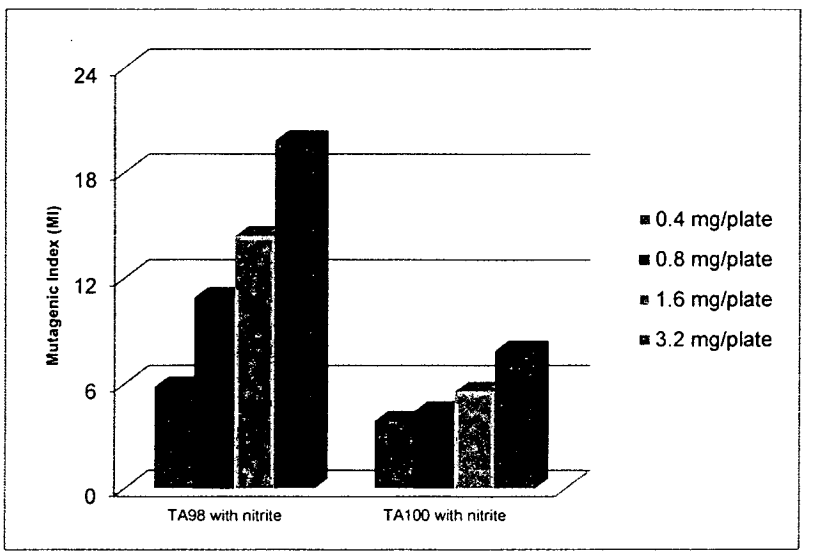


Figure 55 The mutagenic index (MI) induced by the Ben-Cha-Moon-Yai remedy extracts without (55A) and with nitrite (55B) treated 1-aminopyrene on *Salmonella typhimurium* TA98 and TA100 using Ames test.

DNA damage using Comet assay

The DNA migration of cell lymphocytes treated with Ben-Cha-Moon-Yai remedy, five root species extract, positive control and negative control were observed under a fluorescent microscope attached to image capture device with a final magnification of 400x. The degree of DNA damage from all slide were analyzed in terms of tail moment by using the five classes of visual scoring technique, from 0 (no tail) to 4 (almost all DNA tail) give sufficient resolution [134].

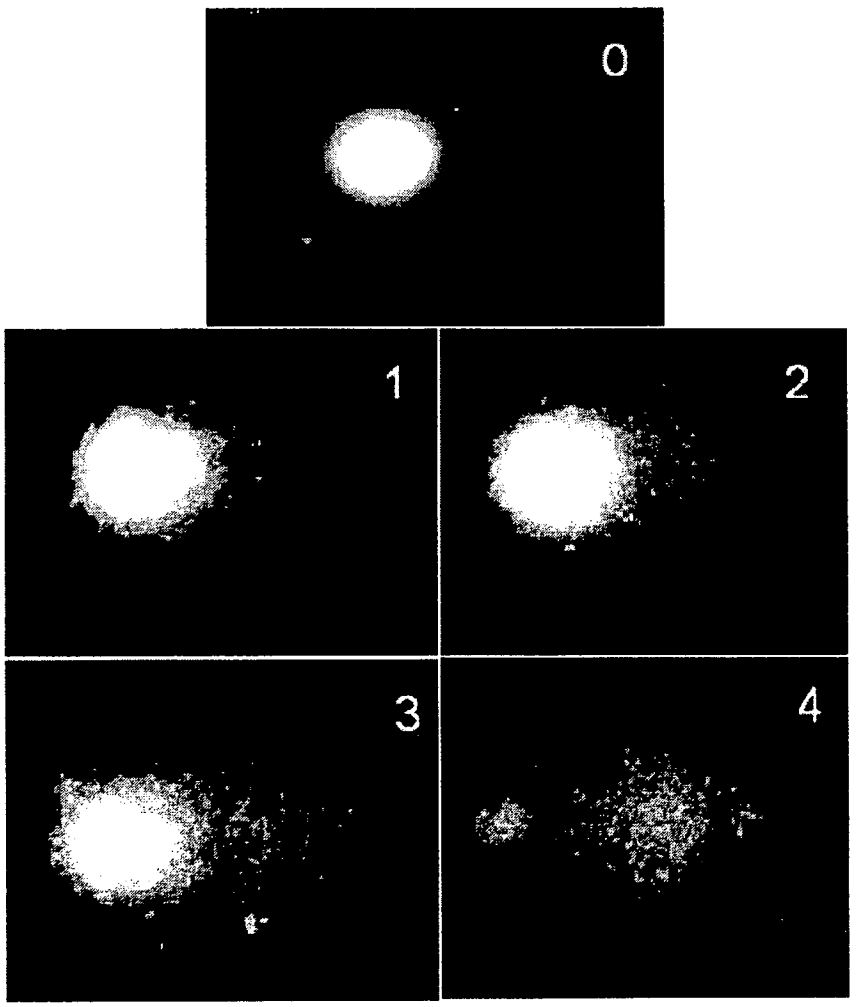


Figure 56 Image of comets from human lymphocytes stained with DAPI with 5 classes of DNA damage used for visual scoring classified by Andrew R. Collin, 2004 [134].

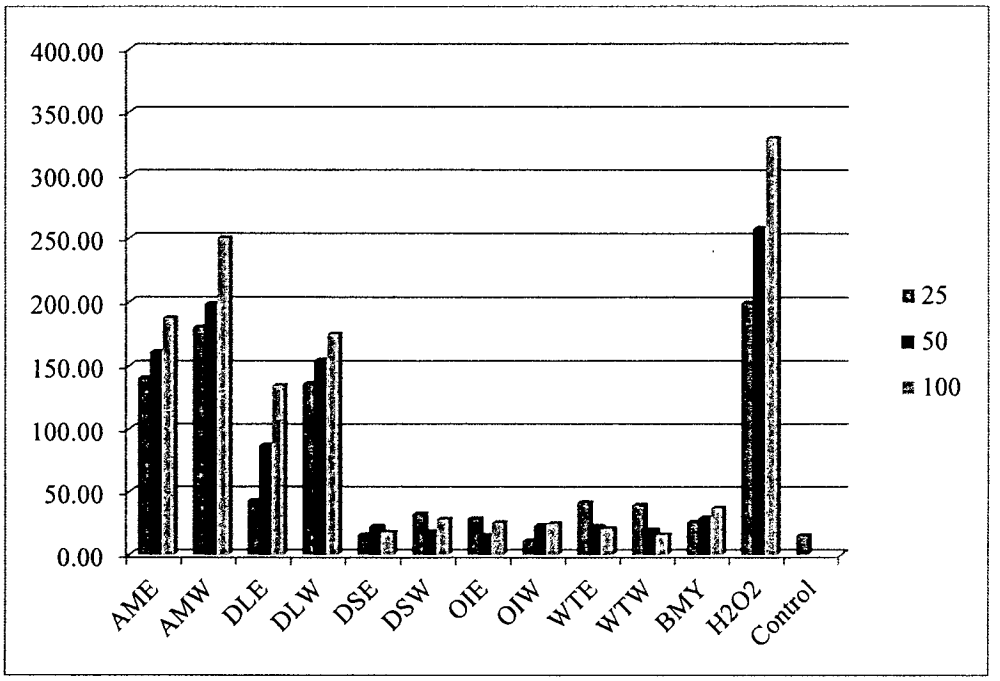


Figure 57 The DNA damage in lymphocytes treated with different concentrations of Ben-Cha-Moon-Yai remedy and five root species or control. The total summing values of the number of comet classification which obtained from the ethanol and water extract of five root species Ben-Cha-Moon-Yai remedy. Abbreviations including: *Aegle marmelos*: AM, *Oroxylum indicum*: OI, *Dimocarpus longan*: DL, *Dolichandrone serrulata*: DS, *Walsura trichostemon*: WT, E: ethanol extract, W: water extract, Ben-Cha-Moon-Yai remedy: BMY.

When 100 comets from each slide were scored, and each comet assigned a value of 0 to 4 according to its class, the total score for the sample gel was between 0 and 400 "arbitrary units". Among five root species and Ben-Cha-Moon-Yai remedy extracts, the result demonstrated that both water and ethanol extract of *A. marmelos* at concentration of 100 μg/ml showed the highest DNA damage in human lymphocytes with the total score of 250.50 and 187.67, respectively. As followed by the human lymphocytes treated with the water and ethanol extract of *D. longan* with presented the total score of 175.33 and 134.67, respectively. Human lymphocytes treated with H_2O_2 showed the highest DNA damage whereas cell treated with phosphase buffer saline showed the lowest DNA damage effects. All samples which induced the DNA damaged in human lymphocytes revealed a dose-dependent relationship between the degree of DNA damage and concentration of sample.

Figure 58 The DNA damage in lymphocytes treated different concentration of Ben-Cha-Moon-Yai remedy and five root species extracts or control.

Figure 58.1 *Aegle marmelos* (Ethanolic extract)

(1A) 25 µg/ml (1B) 50 µg/ml (1C) 100 µg/ml

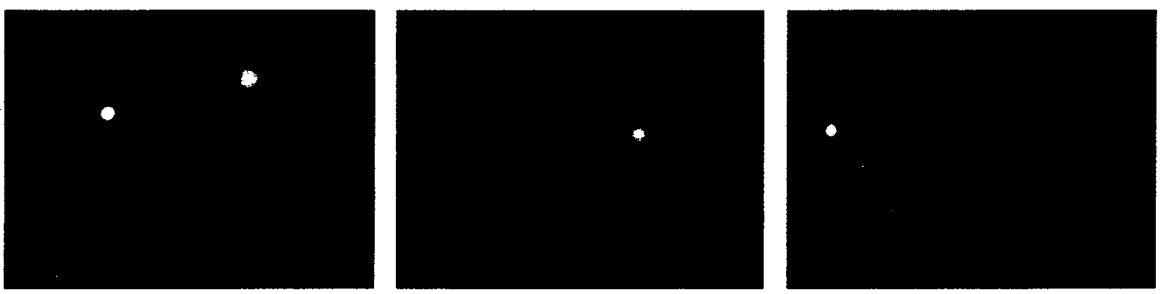


Figure 58.2 *Aegle marmelos* (Water extract)

(2A) 25 µg/ml (2B) 50 µg/ml (2C) 100 µg/ml

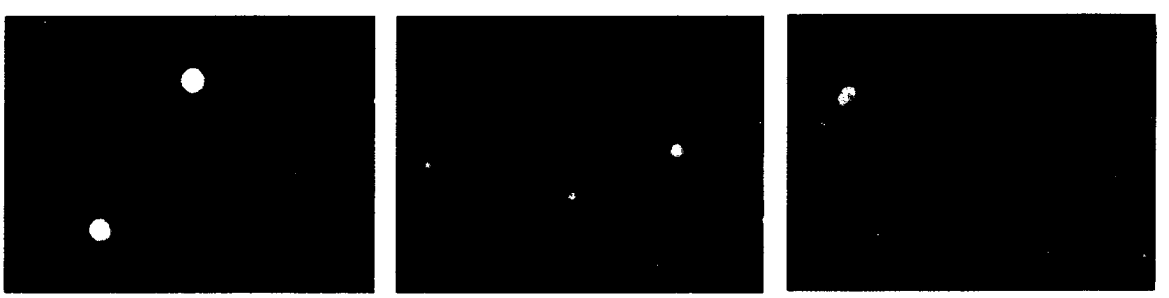


Figure 58.3 *Dimocarpus longan* (Ethanolic extract)

(3A) 25 µg/ml (3B) 50 µg/ml (3C) 100 µg/ml

Figure 58.4 *Dimocarpus longan* (Water extract)

(4A) 25 µg/ml (4B) 50 µg/ml (4C) 100 µg/ml

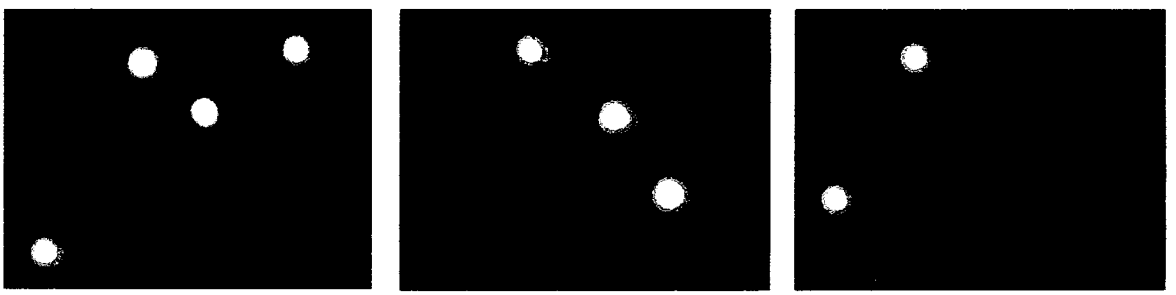


Figure 58.5 *Dolichandrone serrulata* (Ethanolic extract)

(5A) 25 µg/ml (5B) 50 µg/ml (5C) 100 µg/ml

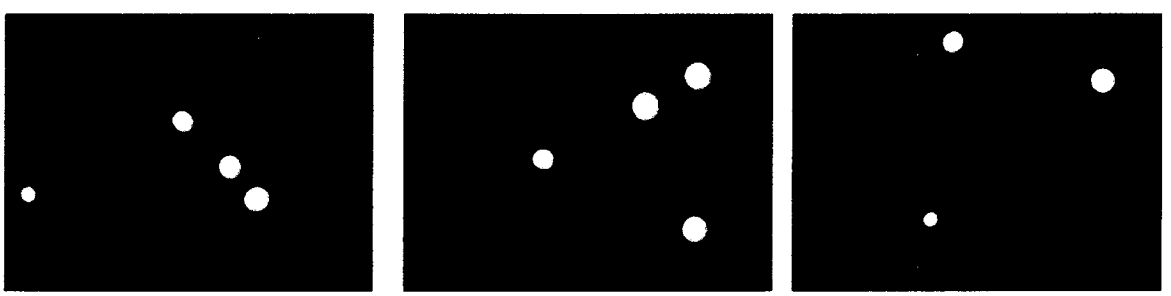


Figure 58.6 *Dolichandrone serrulata* (Water extract)

(6A) 25 µg/ml (6B) 50 µg/ml (6C) 100 µg/ml

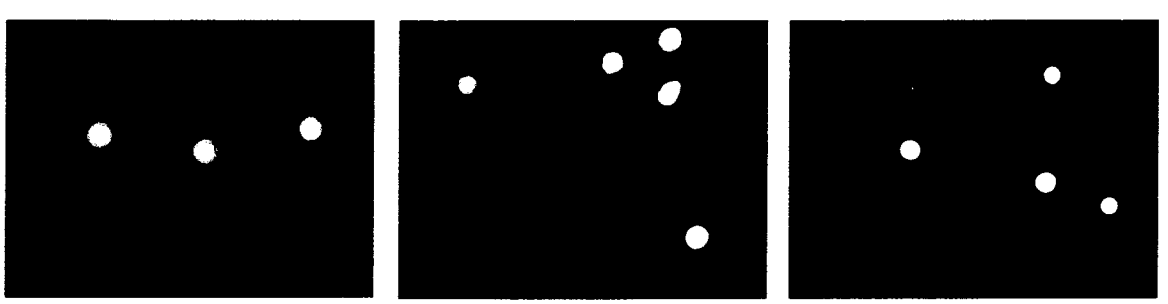


Figure 58.7 *Oroxylum indicum* (Ethanolic extract)

(7A) 25 µg/ml (7B) 50 µg/ml (7C) 100 µg/ml

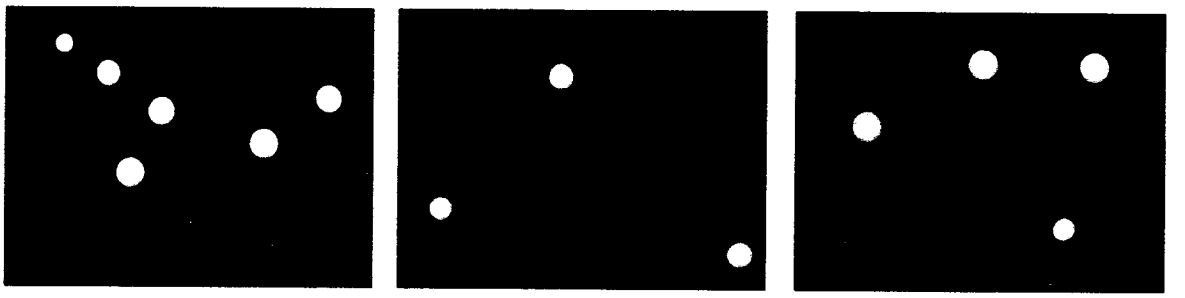


Figure 58.8 *Oroxylum indicum* (Water extract)

(8A) 25 µg/ml (8B) 50 µg/ml (8C) 100 µg/ml

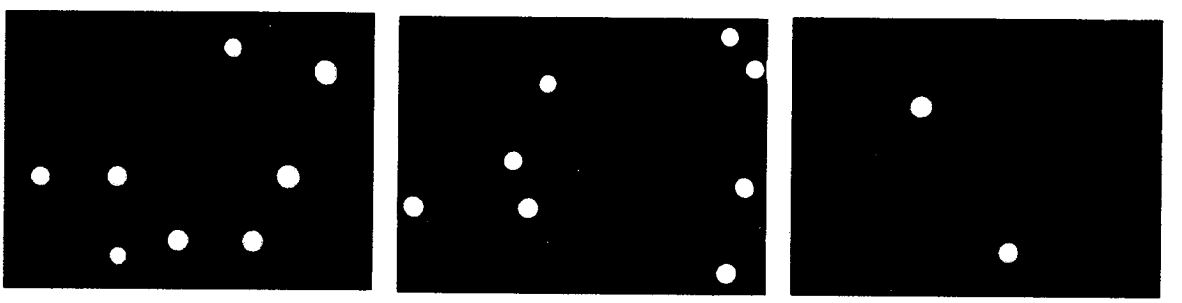


Figure 58.9 *Walsura trichostemon* (Ethanolic extract)

(9A) 25 µg/ml (9B) 50 µg/ml (9C) 100 µg/ml

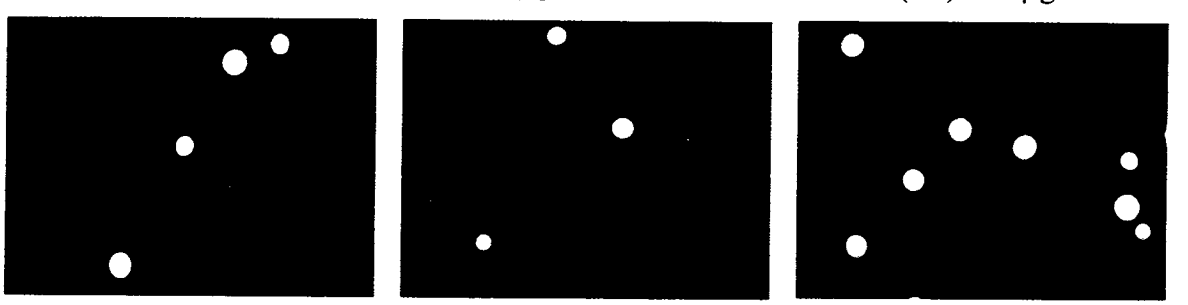


Figure 58.10 *Walsura trichostemon* (Water extract)

(10A) 25 µg/ml (10B) 50 µg/ml (10C)

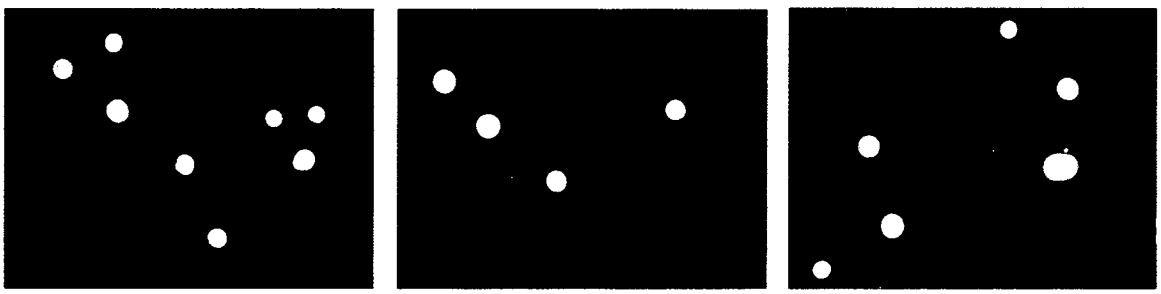


Figure 58.11 Ben-Cha-Moon-Yai remedy

(11A) 25 µg/ml (11B) 50 µg/ml (11C)

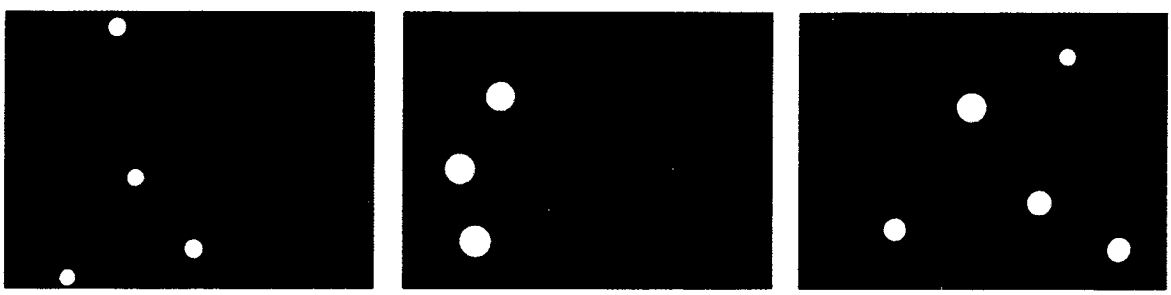


Figure 58.12 Hydrogen Peroxide

(12A) 25 µM (12B) 50 µM (12C) 100 µM

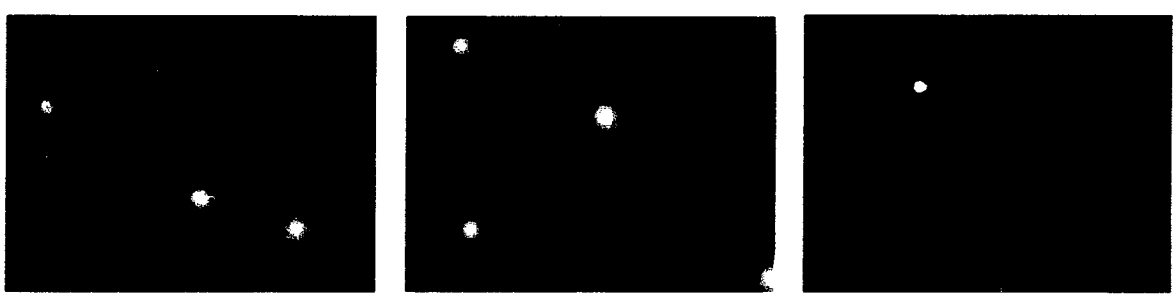
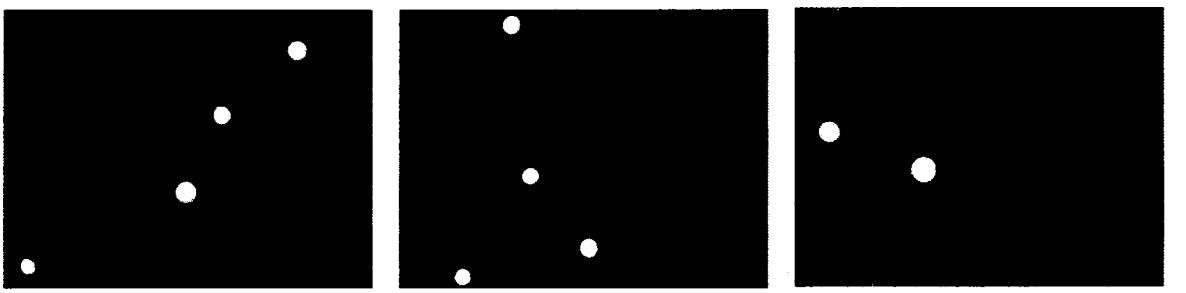


Figure 58.13 Negative Control

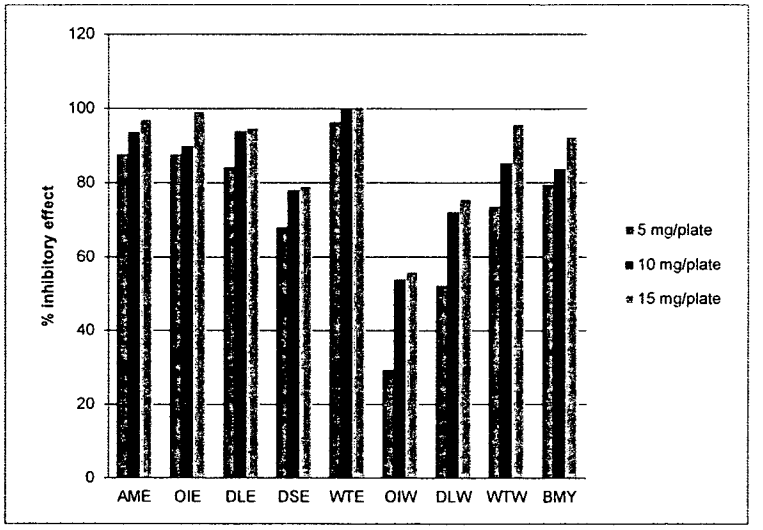
(13A) (13B) (13C)



Antimutagenicity assay

For antimutagenicity assay, all extracts inhibited mutagenicity effect towards *Salmonella typhimurium* strains TA98 and TA100 (figure 59A-59B). The effects were ranged from negligible (0–20% inhibition) to strongly active (>60% inhibition). Only the water extracts of *A. marmelos* and *D. serrulata* were not inhibited the mutagenicity on both strains of *Salmonella typhimurium*. However, the BMY remedy exhibited strong antimutagenicity on both strains of *Salmonella typhimurium*. The percentage of inhibition was increased when the doses were increased. Almost all the roots extract and BMY remedy expressed negligible to strong inhibitory effect (> 60%) on both tester strains. On the other hand, the ethanol extracts of *A. marmelos* (5 mg/plate) had negligible effect (19.5%) on *Salmonella typhimurium* TA 100. The moderate antimutagenic activity was observed on 5, 10 and 15 mg/plate of *O. indicum* water extract on *Salmenella typhimurium* TA98 and 5 mg/plate towards TA100. Whereas, the concentration of 5 mg/plate of the water extract from *D. longan* exhibited the moderate effect on both strains. The moderate (41-60%) to strong antimutagenic (> 60%) effect was observed from the water and ethanol extracts of *O. indicum* toward both strains of *Salmonella typhimurium*, however the water extract at 5 mg/plate exhibited weak inhibitory effect against mutagenicity on strain TA98. All dose of the ethanol extract from *D. serrulata* demonstrated strong antimutagenic effect on both strains, while 5 mg/plate toward strain TA100 exhibited the moderate effect. The strong inhibitory effect was expressed at all concentrations by ethanol extract of *W. trichostemon*, while the weak 20.6% and moderate 51.1% effect were demonstrated when the concentrations of 5 or 10 mg/plate were added to strains TA100, respectively. All of the extracts have dose-related inhibition effect to their mutagenicity of nitrite treated 1-aminopyrene toward *Salmonella typhimurium* strains TA98 and TA100 in the absence of enzyme activating system.

59A (TA98)



59B (TA100)

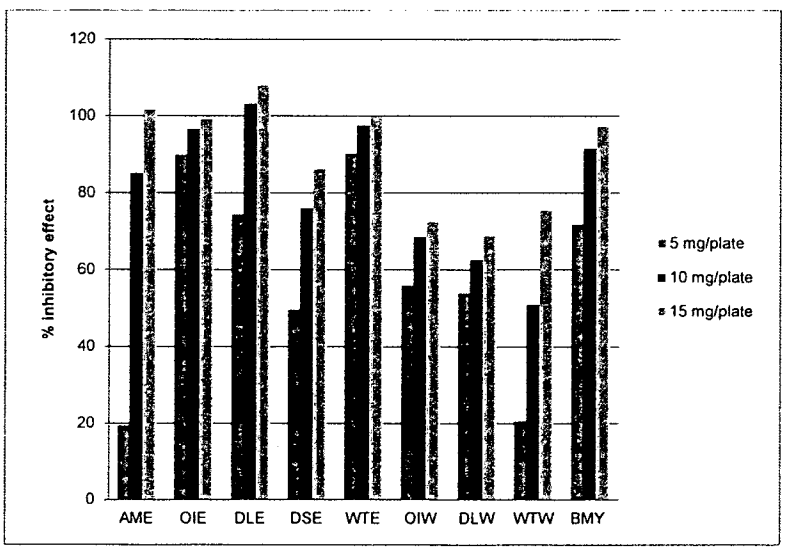


Figure 59 Inhibitory effect of Ben-Cha-Moon-Yai remedy and its components extracts on the mutagenicity of sodium nitrite-treated 1-aminopyrene on *Salmonella typhimurium* strains TA 98 (59A) and TA100 (59B) using Ames test. Abbreviations including: *Aegle marmelos*: AM, *Oroxylum indicum*: OI, *Dimocarpus longan*: DL, *Dolichandrone serrulata*: DS, *Walsura trichostemon*: WT, Ben-Cha-Moon-Yai remedy: BMY, E: ethanol extract, W: water extract.

Antimicrobial activity

The ability of Ben-Cha-Moon-Yai remedy and five root species extracts to inhibit the growth of selected microorganisms were evaluated by agar-well diffusion method and broth microdilution method. The agar-well diffusion test was performed to 13 microorganisms including Gram-positive and negative bacteria and fungi. The active extract which showed the zone inhibition were further investigated in broth microdilution method to obtain MIC, MBC and MFC. The results of the antimicrobial activity have been summarized in table 21-33 showed that the crude ethanol and water extract from five root species and Ben-Cha-Moon-Yai remedy showed the selective activity to prevent the growth of microorganisms.

Table 21 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Staphylococcus aureus*.

Plants/positive controls/ negative control	Solvent extracts	*Staphylococcus aureus*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	9.33±0.58	>2000	>2000
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	13.67±0.58	500	1000
	Water	10.67±0.58	>2000	>2000
Dolichandrone serrulata	Ethanol	8.33±0.58	>2000	>2000
	Water	8.00±0.00	>2000	>2000
Oroxylum indicum	Ethanol	13.00±0.00	1000	>2000
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	7.00±0.00	>2000	>2000
BMY remedy		11.67±0.58	>2000	>2000
Ampicllin		42.00±1.00	0.078	0.156
Amikacin		22.67±0.58	1.25	2.5
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

The results reported in table 1 found that the ethanol extract from the root of *D. longan* showed the highest activity to prevent growth of *Staphylococcus aureus* which exhibited the largest of zone inhibition of 13.67±0.58 mm and the lowest MIC and MBC of 500 and 1000 μg/ml, respectively. The ethanolic extract from *O. indicum* also possessed a large of zone inhibition of 13±0.00, MIC and MBC values of 1000 and > 2000 μg/ml, respectively.

Table 22 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Staphylococcus epidermidis*.

Plants/positive controls/ negative control	Solvent extracts	*Staphylococcus epidermidis*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	NA	NA	NA
	Water	13.67±0.58	1000	>2000
Dolichandrone serrulata	Ethanol	8.67±0.58	>2000	>2000
	Water	NA	NA	NA
Oroxylum indicum	Ethanol	15.00±0.00	1000	>2000
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	7.00±0.00	>2000	>2000
BMY remedy		12.33±0.58	1000	>2000
Ampicllin		23.67±0.58	0.312	0.312
Amikacin		25.67±1.53	1.25	2.5
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

The ethanolic extract of *O. indicum* showed the highest inhibitory effect against *S. epidermidis* which the zone inhibition of 15.00±0.00 mm, followed by the water extract from *D. longan* which provided the zone inhibition of 13.67±0.58 mm. Both active extracts showed the same MIC and MBC values of 1000 and >2000 μg/ml, respectively.

Table 23 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Bacillus cereus*.

Plants/positive controls/ negative control	Solvent extracts	*Bacillus cereus*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	9.33±0.58	>2000	>2000
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dolichandrone serrulata	Ethanol	10.33±0.58	>2000	>2000
	Water	NA	NA	NA
Oroxylum indicum	Ethanol	12.00±0.00	2000	>2000
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	NA	NA	NA
BMY remedy		10.67±0.58	>2000	>2000
Ampicllin		35.00±1.73	0.039	0.039
Amikacin		28.67±0.58	0.312	0.625
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

The ethanolic extract from the root of *O. indicum* showed the largest of zone inhibition of 12.00±0.00 mm against *B. cereus* and the lowest MIC and MBC of 2000 and >2000 μg/ml, respectively.

Table 24 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Bacillus subtilis*.

Plants/positive controls/ negative control	Solvent extracts	*Bacillus subtilis*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	7.67±0.58	>2000	>2000
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	14.33±0.58	>2000	>2000
	Water	12.67±0.58	>2000	>2000
Dolichandrone serrulata	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Oroxylum indicum	Ethanol	11.67±0.58	1000	>2000
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	NA	NA	NA
BMY remedy		9.67±0.58	>2000	>2000
Ampicllin		17.00±0.00	5	10
Amikacin		24.67±1.53	0.625	0.625
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

The ethanolic extract from *D. longan* root exhibited the largest on zone inhibition of 14.33±0.58 against *B. subtilis* whereas the lowest MIC was found in the ethanolic extract of *O. indicum* which MIC and MBC values of 1000 and >2000 μg/ml, respectively.

Table 25 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Micrococcus luteus*.

Plants/positive controls/ negative control	Solvent extracts	*Micrococcus luteus*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	11.00±1.00	>2000	>2000
	Water	9.67±0.58	>2000	>2000
Dimocarpus loDngan	Ethanol	15.00±1.00	>2000	>2000
	Water	11.00±0.00	>2000	>2000
Dolichandrone serrulata	Ethanol	9.33±0.58	>2000	>2000
	Water	12.33±0.58	>2000	>2000
Oroxylum indicum	Ethanol	19.00±0.00	1000	2000
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	10.00±0.00	>2000	>2000
	Water	10.00±0.00	>2000	>2000
BMY remedy		15.00±0.00	1000	2000
Ampicllin		49.00±1.00	0.156	0.312
Amikacin		29.00±1.00	1.25	2.5
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

The ethanolic extract from the root of *O. indicum* showed the largest of zone inhibition of 19.00±0.00 mm followed by the and the MIC, MBC values of 1000 and 2000 μg/ml. Ben-Cha-Moon-Yai remedy reported the zone inhibition of 15.00±0.00 mm and the MIC, MBC values of 1000 and 2000 μg/ml, respectively.

Table 26 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Enterobacter aerogenes*.

Plants/positive controls/ negative control	Solvent extracts	*Enterobacter aerogenes*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dolichandrone serrulata	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Oroxylum indicum	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	NA	NA	NA
BMY remedy		NA	NA	NA
Ampicllin		11.00±0.00	>10	>10
Amikacin		16.33±0.58	1.25	5
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

All crude extracts were not inhibited the growth of *E. aerogenes* in the agar-well diffusion method. The lowest of inhibition concentration and the lowest of bactericidal concentration was found in Amikacin of 1.25 and 5 μg/ml.

Table 27 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Escherichia coli*.

Plants/positive controls/ negative control	Solvent extracts	*Escherichia coli*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dolichandrone serrulata	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Oroxylum indicum	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	NA	NA	NA
BMY remedy		NA	NA	NA
Ampicllin		23.00±0.00	5	5
Amikacin		19.00±0.00	1.25	1.25
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

All crude extracts were not inhibited the growth of *E. coli* in the agar-well diffusion method. The lowest of inhibition concentration and the lowest of bactericidal concentration was found in Amikacin of 1.25 and 1.25 μg/ml.

Table 28 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Pseudomonas aeruginosa*.

Plants/positive controls/ negative control	Solvent extracts	*Pseudomonas aeruginosa*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dolichandrone serrulata	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Oroxylum indicum	Ethanol	10.67±0.58	>2000	>2000
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	NA	NA	NA
BMY remedy		NA	NA	NA
Ampicllin		NA	NA	NA
Amikacin		17.33±0.58	1.25	1.25
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

The ethanolic extract of *O. indicum* showed the largest of zone inhibition of 10.67±0.58 mm against *P. aeruginosa* and the MIC and MBC values of >2000 μg/ml.

Table 29 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Salmonella typhi*.

Plants/positive controls/ negative control	Solvent extracts	*Salmonella typhi*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dolichandrone serrulata	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Oroxylum indicum	Ethanol	10.33±0.58	>2000	>2000
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	NA	NA	NA
BMY remedy		7.33±0.58	>2000	>2000
Ampicllin		27.00±0.00	1.25	1.25
Amikacin		20.33±0.58	1.25	2.5
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

The ethanolic extract of *O. indicum* showed the largest of zone inhibition of 10.33±0.58 mm against *S. typhi* and the MIC and MBC values of >2000 μg/ml.

Table 30 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Salmonella typhimurium*.

Plants/positive controls/ negative control	Solvent extracts	*Salmonella typhimurium*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dolichandrone serrulata	Ethanol	NA	NA	NA
	Water	9.00±0.00	>2000	>2000
Oroxylum indicum	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	NA	NA	NA
BMY remedy		10.00±0.00	>2000	>2000
Ampicllin		31.67±1.15	0.625	0.625
Amikacin		23.67±1.53	0.625	0.625
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

The largest of zone inhibition was found in the Ben-Cha-Moon-Yai remedy of 10.00±0.00 mm and MIC, MBC values of > 2000 μg/ml.

Table 31 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Shigella spp.*

Plants/positive controls/ negative control	Solvent extracts	*Shigella spp.*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dolichandrone serrulata	Ethanol	8.00±0.00	>2000	>2000
	Water	9.33±0.58	>2000	>2000
Oroxylum indicum	Ethanol	9.00±0.00	>2000	>2000
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	NA	NA	NA
BMY remedy		11.33±0.58	>2000	>2000
Ampicllin		24.00±2.65	2.5	5
Amikacin		22.00±1.00	1.25	2.5
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

Ben-Cha-Moon-Yai remedy showed the largest of zone inhibition of 11.33±0.58 mm and MIC, MBC values of > 2000 μg/ml.

Table 32 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Candida albicans*.

Plants/positive controls/ negative control	Solvent extracts	*Candida albicans*		
		Inhibition zone (mm)	MIC (μg/ml)	MFC (μg/ml)
Aegle marmelos	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dolichandrone serrulata	Ethanol	NA	NA	NA
	Water	8.33±0.58	500	500
Oroxylum indicum	Ethanol	16.33±0.58	>2000	>2000
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	NA	NA	NA
BMY remedy		NA	NA	NA
Ampicllin		NA	NA	NA
Amikacin		NA	NA	NA
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

The ethanolic extract of *O. indicum* showed the largest of zone inhibition of 16.33±0.58 whereas the lowest inhibition concentration and the lowest fungicidal concentration was found in the water extract of *D. serrulata* of 500 μg/ml

Table 33 The antimicrobial activities of Ben-Cha-Moon-Yai remedy and five root species extracts at 4 mg/ml against *Saccharomyces cerevisiae*.

Plants/positive controls/ negative control	Solvent extracts	*Saccharomyces cerevisiae*		
		Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Aegle marmelos	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dimocarpus longan	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Dolichandrone serrulata	Ethanol	NA	NA	NA
	Water	NA	NA	NA
Oroxylum indicum	Ethanol	11.67±0.58	500	2000
	Water	NA	NA	NA
Walsura trichostemon	Ethanol	NA	NA	NA
	Water	NA	NA	NA
BMY remedy		NA	NA	NA
Ampicllin		NA	NA	NA
Amikacin		NA	NA	NA
DMSO		NA	NA	NA

Mean ± SD, NA = no activity, each experiment was done in triplicate.

Only the ethanolic extract from the root of *O. indicum* showed the inhibitory effect against the growth of *S. cerevisiae* which provided the zone inhibition of 11.67±0.58 mm and the MIC and MBC of 500 and 2000 μg/ml.

Free radical scavenging assay (DPPH assay)

Results demonstrated that the ethanolic extract of the *Dimocarpus longan* root showed the highest radical scavenging activity with IC_{50} of 9.3 μg/ml, followed by water extract of *Walsura trichostemon* showed IC_{50} of 16.1 μg/ml. Ben-Cha-Moon-Yai remedy extract showed the IC_{50} of 81.9 μg/ml. The water extract of *Oroxylum indicum* had the lowest radical-scavenging activity (IC_{50} 409.31 μg/ml).

Table 34 Inhibition concentration of DPPH scavenging activity (IC_{50}) of the extracts of Ben-Cha-Moon-Yai remedy and five root species extracts.

Plant name	**DPPH scavenging activity Mean IC_{50} (μg/ml)**	
	Ethanol extract	**Water extract**
Aegle marmelos	61.3	380.0
Dolichandrone serrulata	87.8	338.5
Dimocarpus longan	**9.3**	40.8
Oroxylum indicum	61.3	380.0
Walsura trichostemon	23.8	**16.1**
Ben-Cha-Moon-Yai remedy	81.9	
BHT	22.3	
Quercetin	9.8	

Lipid peroxidation testing using β-carotene bleaching assay

The ethanolic extract of *D. longan* showed highest ability to prevent the bleaching of β-carotene followed by the ethanolic extract of *A. marmelos*, Ben-Cha-Moon-Yai remedy, *W. trichostemon*, *D. serrulata*, and *O. indicum*, respectively. While, the water extract of *D. longan* showed higher ability to prevent the bleaching of β-carotene followed by the water extract of *W. trichostemon*, *O. indicum*, *D. serrulata*, and *A. marmelos*, respectively. All extracts had lower antioxidant activities than BHT and quercetin.

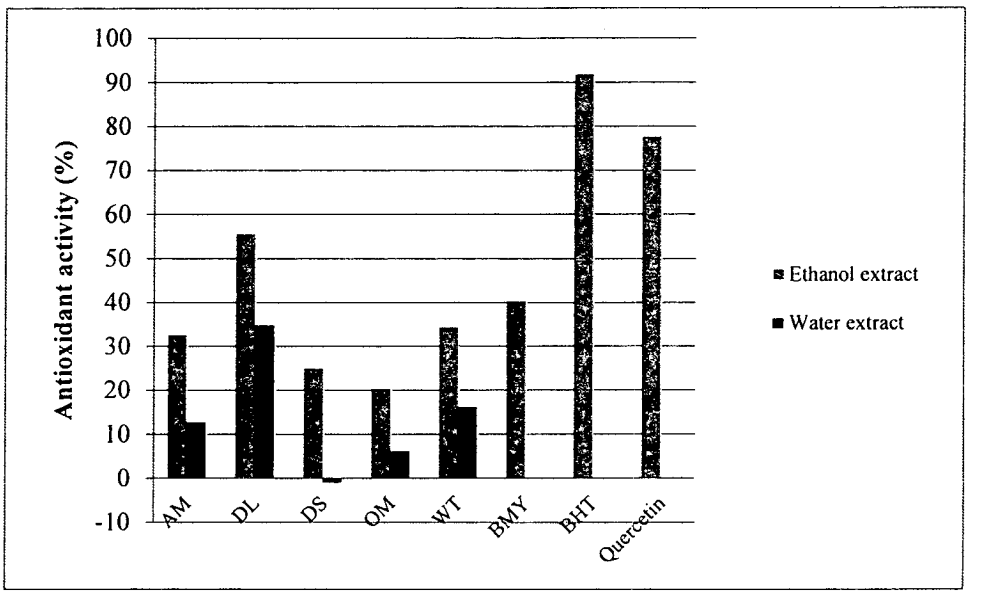


Figure 60 The antioxidant activity of five root species and Ben-Cha-Moon-Yai remedy extract against β-carotene bleaching assay. Abbreviations including: *Aegle marmelos*: AM, *Oroxylum indicum*: OI, *Dimocarpus longan*: DL, *Dolichandrone serrulata*: DS, *Walsura trichostemon*: WT, Ben-Cha-Moon-Yai remedy: BMY.

Figure 60A

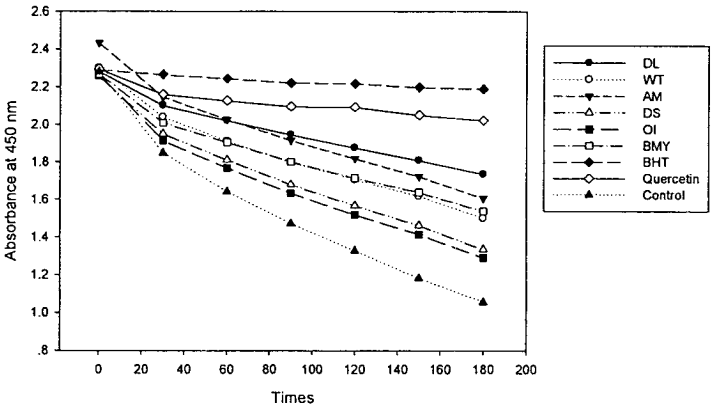


Figure 60B

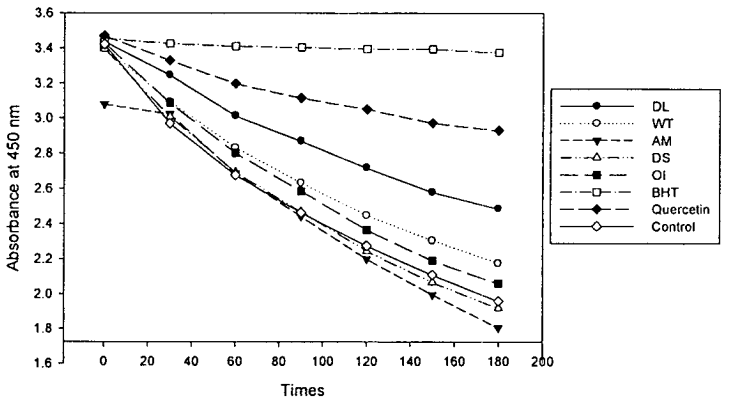


Figure 60 Changes of the absorbance at 450 nm with time for the ethanol (60A) and water (60B) extracts (1 mg/ml) in β -carotene bleaching assay. Abbreviations including: *Aegle marmelos*: AM, *Oroxylum indicum*: OI, *Dimocarpus longan*: DL, *Dolichandrone serrulata*: DS, *Walsura trichostemon*: WT, Ben-Cha-Moon-Yai remedy: BMY.

Nitric oxide scavenging assay using Griess reagent assay

Results demonstrated that the ethanolic extract from *D. longan* expressed the high potential on nitric oxide scavenging activity with IC_{50} of 23 μg/ml, followed by the ethanolic extract of *W. trichostemon* with IC_{50} of 25 μg/ml, while the Ben-Cha-Moon-Yai remedy extract exhibited the IC_{50} of 657 μg/ml.

Table 35 Inhibition concentration of nitric oxide scavenging activity (IC_{50}) of Ben-Cha-Moon Yai remedy and five root species extracts.

Plant name	**NO scavenging activity Mean IC_{50} (μg/ml)**	
	Ethanol extract	**Water extract**
Oroxylum indicum	716	3521
Aegle marmelos	883	1531
Dimocarpus longan	**23**	367
Walsura trichostemon	**25**	933
Dolichandrone serrulata	161	3577
Ben Cha Moon Yai remedy	**657**	
Quercetin	9.17	

Total phenolic contents

The quantification of total phenolic content by Folin-Ciocalteu reagent demonstrated that the water extract of *D. longan* showed the highest of total phenolic content resulting of 10.033±0.218 mg catechin hydrate/ 500 mg extract, followed by the water extract of *W. trichostemon* which resulting 8.864±0.348 mg catechin hydrate/ 500 mg extract calculated from the calibration curve of catechin dydrate equation: $y = 0.007x + 0.022$ ($R^2 = 0.989$).

Table 36 Total phenolic content from the water extract of five root species in Ben-Cha-Moon-Yai remedy at 500 mg/ml.

Plant name	**Total phenolic contents (µg CE/ 500 mg/ml of the extract)**	
	Mean	**SD**
Oroxylum indicum	5.55	0.12
Aegle marmelos	6.06	1.17
Dimocarpus longan	**10.03**	0.22
Walsura trichostemon	**8.86**	0.35
Dolichandrone serrulata	5.82	0.02

Antipyretic activity

Effects of an extract from Ben-Cha-Moon-Yai remedy on LPS-induce fever

Lipopolysaccharide (LPS; 50 μg/kg) injected intramuscularly significantly (p<0.001) produced a time-dependent increase in rectal temperature in vehicle pretreated rats starting from 1 hr and this effect was maintained for 7 hr after LPS injection. The maximum increase in rectal temperature was reached at 2 hr (0.89°C) giving a maximum observed mean rectal temperature of 38.69 ± 0.14°C after which there was a decrease (Figure 61). At the same time, the mean rectal temperature of normothermic rats was 37.80 ± 0.15°C. Thus, LPS significantly (p<0.001) increased the rectal temperature (Figure 61).

Acetylsalicylic acid (ASA; 300 mg/kg) significantly (p<0.05) reduced the increased rectal temperature produced by LPS over a period of 7 hr with a maximum reduction at 2 hr. The mean rectal temperature produced by LPS in the presence of ASA was reduced to 37.68 ± 0.23°C (Figure 61).

BMY at the dose of 125 mg/kg significantly (p<0.01) attenuated the increase in rectal temperature produced by LPS starting at 2 hr and the effect was maintained for the full 7 hr with a maximum reduction at 3 hr after LPS injection. BMY at the dose of 250 mg/kg significantly reduced LPS-induced increase in rectal temperature at 2 and 3 hr (p<0.05 and p<0.01, respectively) with a maximum reduction at 3 hr after LPS injection. BMY at the dose of 500 mg/kg significantly (p<0.01) attenuated the increase in rectal temperature produced by LPS starting at 1 hr and the effect was maintained for the full 7 hr with a maximum reduction at 3 hr after LPS injection (Figure 62).

Effects of an extracts from five root species in Ben-Cha-Moon-Yai remedy on LPS-induce fever

1. The root extract of *Aegle marmelos* (AM)

AM at the doses of 25 and 50 mg/kg significantly ($p<0.05$) reduced LPS-induced increase in rectal temperature at 2 and 3 hr after LPS injection and both doses showed a maximum reduction at 3 hr. AM at the dose of 200 mg/kg significantly ($p<0.05$) reduced LPS-induced increase in rectal temperature at 3 hr after LPS injection. AM at the dose of 400 mg/kg significantly ($p<0.01$) reduced the increased rectal temperature produced by LPS over a period of 2-7 hr with a maximum reduction at 3 hr (Figure 63).

2. The root extract of *Dolichandrone serrulata* (DS)

All doses of DS could not reduce LPS-induced increase in rectal temperature (Figure 16). All doses of DS did not show antipyretic effect (Figure 64).

3. The root extract of *Dimocarpus longan* (DL)

DL at the doses of 50,100 and 200 mg/kg significantly ($p<0.05$) reduced LPS-induced increase in rectal temperature over a period of 2-3 hr with a maximum reduction at 2, 2 and 2 hr, respectively (Figure 65) . All doses of DL did not show antipyretic effect.

4. The root extract of *Oroxylum indicum* (OI)

OI at the dose of 25 mg/kg significantly ($p<0.05$) reduced LPS-induced increase in rectal temperature at 2 and 3 hr with a maximum reduction at 2 hr. OI at the dose of 50 mg/kg significantly ($p<0.05$) reduced LPS induced increase in rectal temperature at 3 hr. OI at the dose of 100 mg/kg significantly reduced LPS-induced increase in rectal temperature at 5, 6 and 7 hr ($p<0.05$, $p<0.05$ and $p<0.01$) with a maximum reduction at 7 hr. OI at the dose of 200 mg/kg significantly reduced LPS induced increase in rectal temperature at 2 and 3 hr after LPS injection ($p<0.01$ and $p<0.05$) with a maximum reduction at 2 hr. OI at the dose of 400 mg/kg significantly

reduced LPS induced increase in rectal temperature at 2, 3, 5, 6, and 7 hr after LPS injection ($p<0.01$, $p<0.01$, $p<0.05$, $p<0.01$ and $p<0.01$, respectively) with a maximum reduction at 3 hr (Figure 66). OI at the dose of 400 mg/kg seemed to have the highest antipyretic efficacy.

5. The root extract of *Walsura trichostemon* (WT)

WT at the dose of 25 mg/kg significantly ($p<0.05$) reduced LPS-induced increase in rectal temperature at 4-7 hr after LPS injection with a maximum reduction at 7 hr. WT at the dose of 50 mg/kg significantly ($p<0.05$) reduced LPS-induced increase in rectal temperature at 4 and 7 hr. WT at the dose of 100 mg/kg significantly ($p<0.05$) reduced LPS-induced increase in rectal temperature at 2, 3, 4, 6 and 7 hr with a maximum reduction at 4 hr. WT at the dose of 200 mg/kg significantly ($p<0.05$) reduced LPS-induced increase in rectal temperature at 4-7 hr with a maximum reduction at 5 hr (Figure 67).

Lipopolysaccharide-induced Fever in Rats

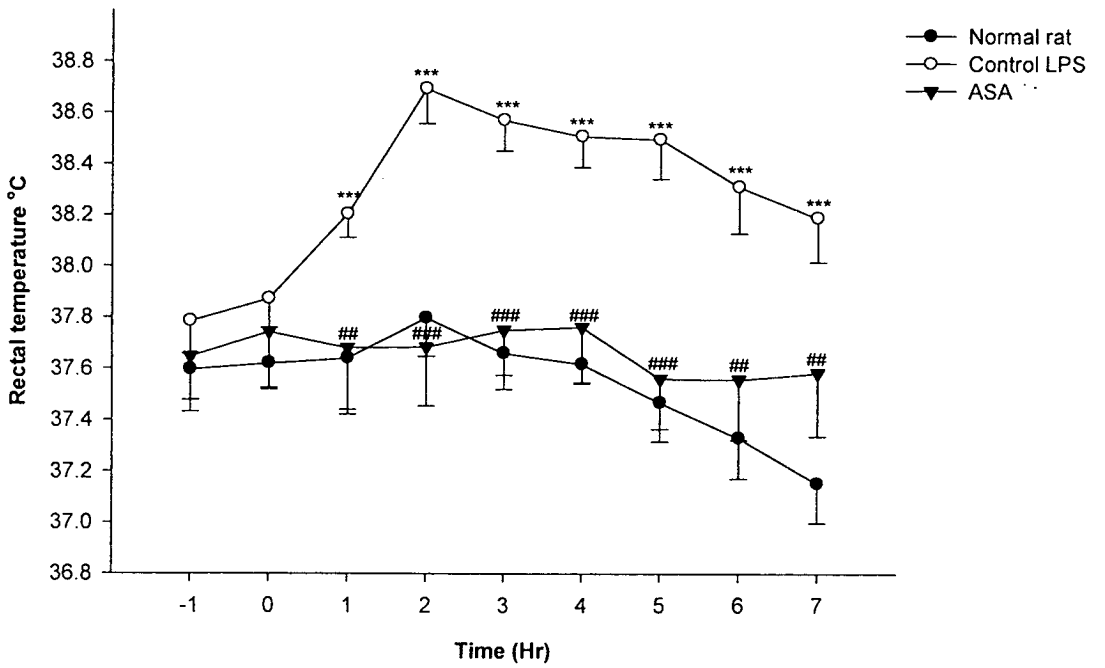


Figure 61 Changes in rectal temperature after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg). Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. Normal rats were received 0.9% NSS injection instead of LPS. N=6 for all groups. $^{***}p<0.001$ significantly different compared to normal rat values for the corresponding hour. $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ significantly different compared to control LPS values at the corresponding hour.

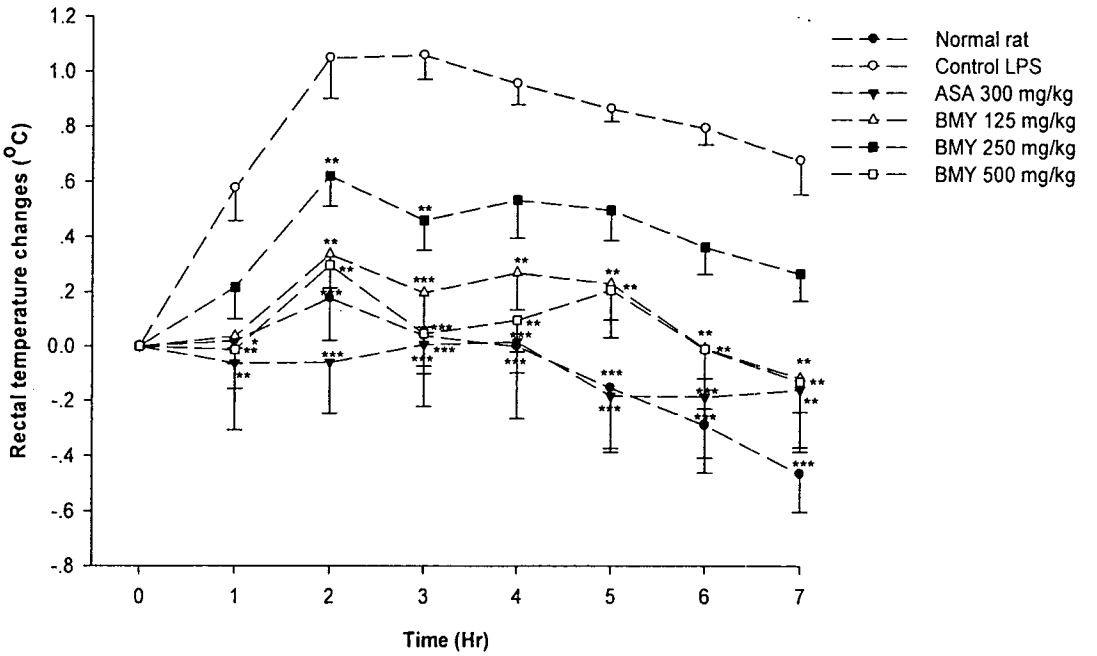


Figure 62 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ significantly different compared to control LPS values at the corresponding hour.

The root extract of *Aegle marmelos* (AM)

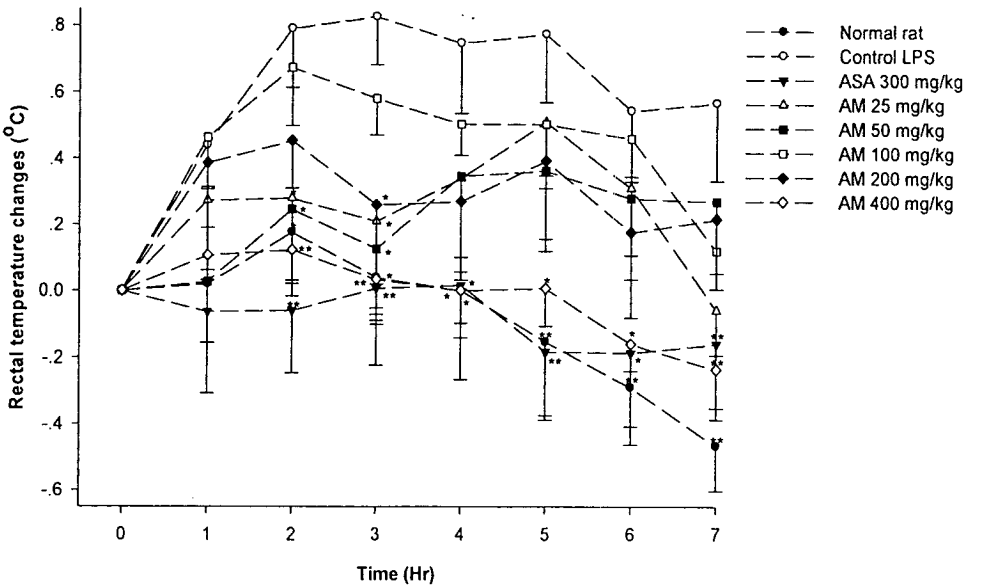


Figure 63 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Aegle marmelos* (AM; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. *$p<0.05$ and **$p<0.01$ significantly different compared to control LPS values at the corresponding hour.

The root extract of *Dolichandrone serrulata* (DS)

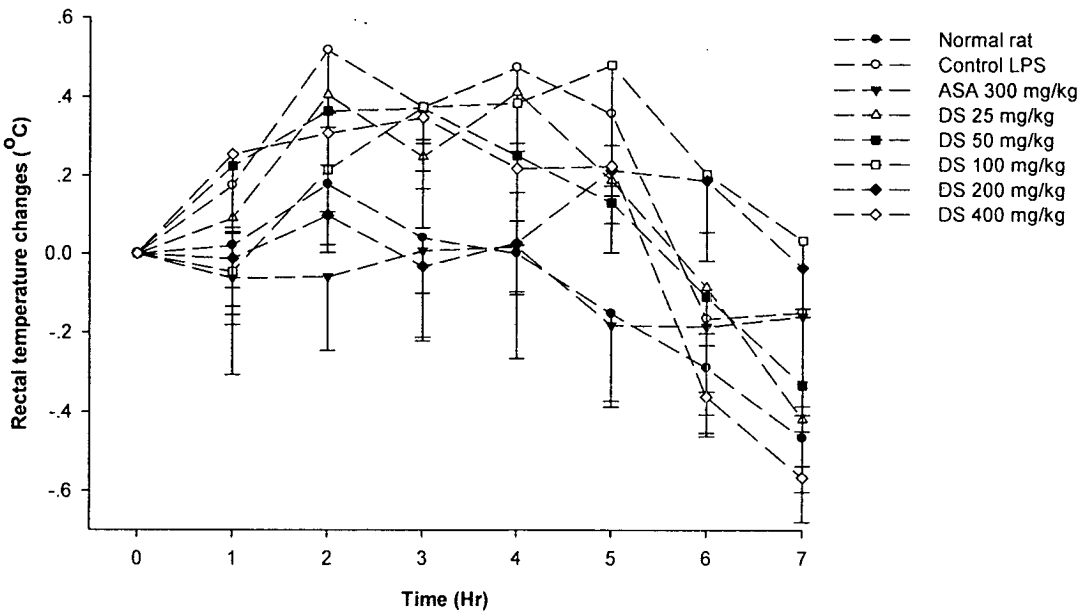


Figure 64 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Dolichandrone serrulata* (DS; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups.

The root extract of *Dimocarpus longan* (DL)

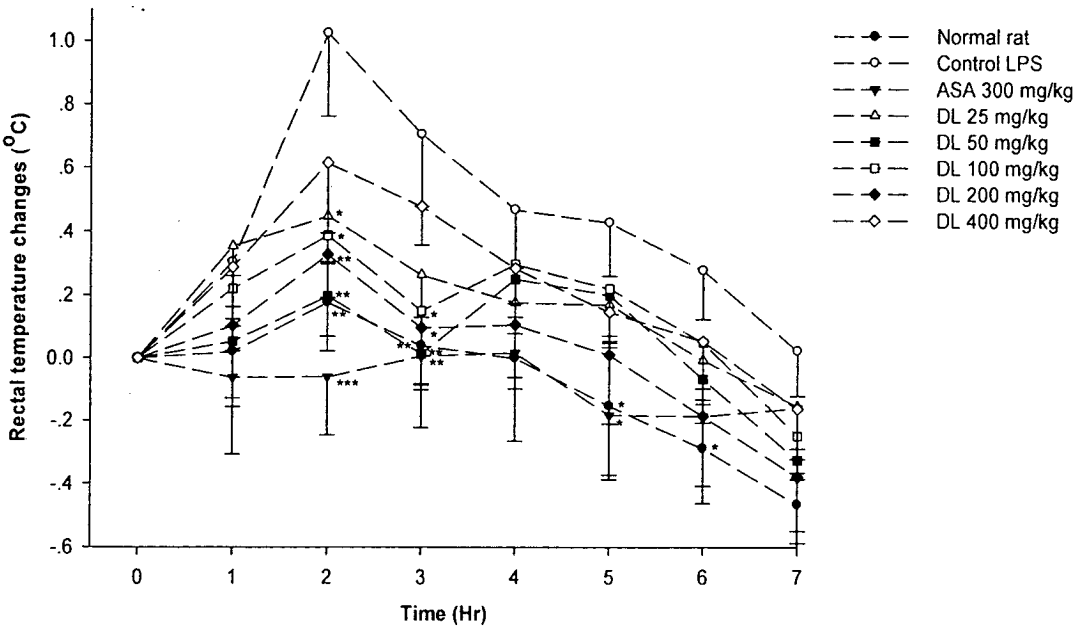


Figure 65 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract *of Dimocarpus longan* (DL; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ significantly different compared to control LPS values at the corresponding hour.

The root extract of *Oroxylum indicum* (OI)

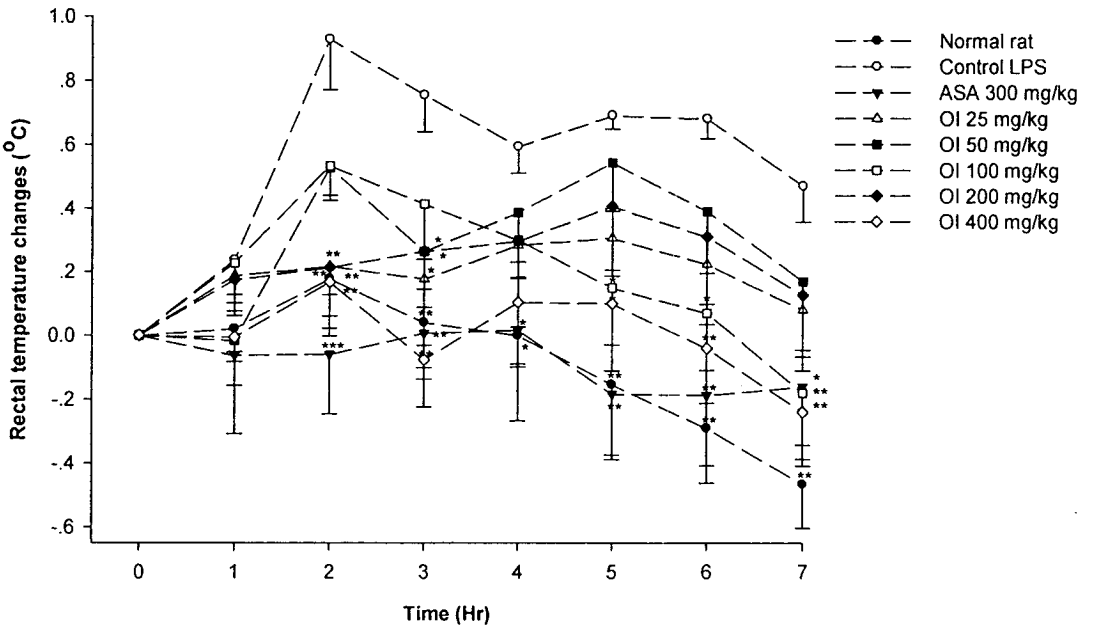


Figure 66 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Oroxylum indicum* (OI; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ significantly different compared to control LPS values at the corresponding hour.

The root extract of *Walsura trichostemon* (WT)

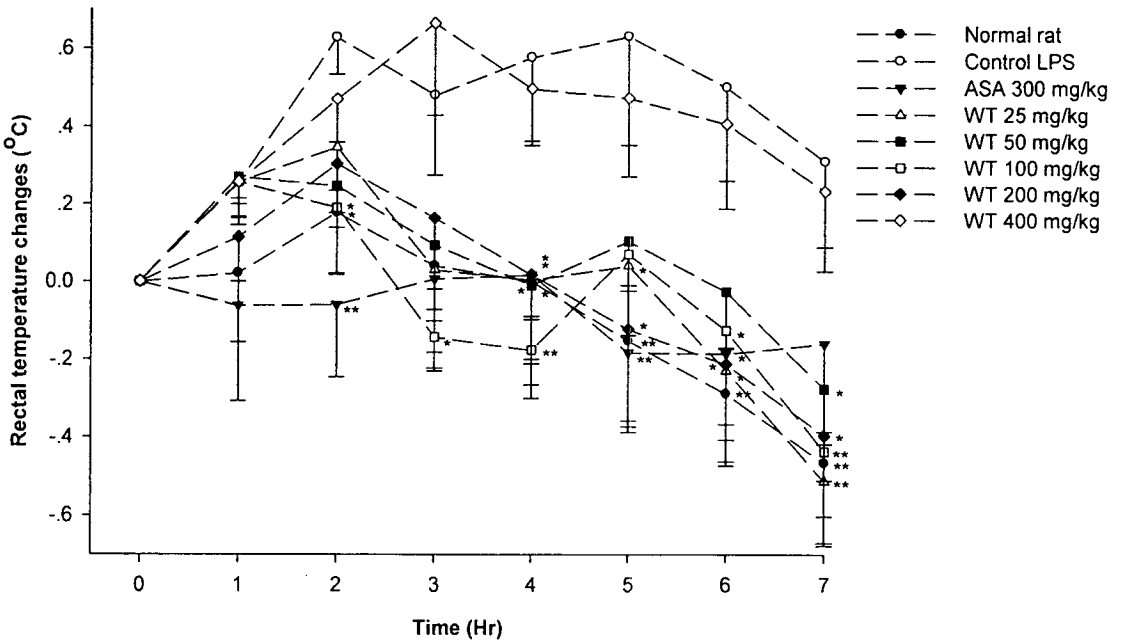


Figure 67 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Walsura trichostemon* (WT; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. *p<0.05, and **p<0.01 significantly different compared to control LPS values at the corresponding hour.

Anti-inflammatory activity

Carrageenan-induced paw edema in mice

The anti-inflammatory effects of Ben-Cha-Moon-Yai remedy and five root species extracts were initially evaluated in the carrageenan-induced paw edema in mice. Each mouse was orally pre-treated with 2% Tween 80, indomethacin (IND; 10 mg/kg), various doses of BMY (125, 250, 500 mg/kg) or AM, OI, DL, DS, WT (25, 50, 100, 200, 400 mg/kg). IND 10 mg/kg was used as a positive control. The result demonstrated that IND was the most potent drug with significantly decreased paw edema after carrageenan administration by 40.48%, 56.16%, 69.35% and 72.22% at 2, 3, 4 and 5 hr, respectively when compared to 2% Tween 80.

BMY 125 mg/kg significantly ($p<0.05$) decreased paw edema and produced an inhibition of paw edema of 32.91% at 3 hr after carrageenan administration compared with that of 2% Tween 80. BMY at the dose of 250 mg/kg significantly ($p<0.001$) decreased paw edema at 3 and 4 hr compared with that of 2% Tween 80 and produced a maximum inhibition of paw edema of 51.61% at 4 hr. The highest dose of BMY (500 mg/kg) significantly ($p<0.001$, $p<0.001$ and $p<0.05$, respectively) decreased paw edema at 3, 4 and 5 hr compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 65.77% at 3 hr. The extracts demonstrated a dose-dependent by inhibitory effect against paw edema in mice at 3 hr. BMY at 500 mg/kg produced high anti-inflammatory effect than IND (Table 37).

AM at the dose of 400 mg/kg significantly ($p<0.05$) decreased paw edema at 3 and 4 hr after carrageenan administration compared to 2% Tween 80 and produced an inhibition of paw edema of 23.93% at 4 hr. All doses of AM (25-400 mg/kg) produced less anti-inflammatory effect than IND (Table 386).

DL at the dose of 200 mg/kg significantly ($p<0.05$) decreased paw edema at 3, 4 and 5 hr after carrageenan administration compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 34.85% at 4 hr. DL at the dose of 400 mg/kg significantly ($p<0.05$, $p<0.01$ and $p<0.05$, respectively) decreased paw edema at 3, 4 and 5 hr compared to 2% Tween 80 and produced a maximum inhibition of paw

edema of 39.39% at 4 hr. All doses of DL (25-400 mg/kg) produced less anti-inflammatory effect than IND (Table 39).

DS at the dose of 200 mg/kg significantly ($p<0.05$) decreased paw edema at 4 hr after carrageenan administration compared to 2% Tween 80 and produced an inhibition of paw edema of 29.69%. DS at the dose of 400 mg/kg significantly ($p<0.05$) decreased paw edema at 3 and 4 hr after carrageenan administration compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 34.38% at 4 hr. All doses of DS (25-400 mg/kg) produced less anti-inflammatory effect than IND (Table 40).

OI at the dose of 200 mg/kg significantly ($p<0.05$) decreased paw edema at 3 and 4 hr after carrageenan administration compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 26.18% at 4 hr. OI at the dose of 400 mg/kg significantly ($p<0.05$, $p<0.01$ and $p<0.05$, respectively) decreased paw edema at 3, 4 and 5 hr compared to 2% Tween 80 and showed a maximum inhibition of paw edema of 32.14% at 3 hr after carrageenan administration. All doses of OI (25-400 mg/kg) produced less anti-inflammatory effect than IND (Table 41).

WT at the dose of 25 mg/kg significantly ($p<0.01$ and $p<0.05$, respectively) decreased paw edema at 3 and 4 hr after carrageenan administration compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 36.76% at 3 hr. WT at doses of 50 and 100 mg/kg significantly ($p<0.01$) decreased paw edema at 3 and 4 hr compared to 2% Tween 80 and produced a similar maximum inhibition of paw edema of 42.65% at 3 hr. WT at the dose of 200 mg/kg significantly ($p<0.001$ and $p<0.01$, respectively) decreased paw edema at 3 and 4 hr compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 47.06% at 3 hr. WT at the dose of 400 mg/kg significantly ($p<0.001$, $p<0.001$ and $p<0.05$, respectively) decreased paw edema at 3, 4 and 5 hr compared to 2% Tween 80 administration and produced a maximum inhibition of paw edema of 48.53% at 3 hr. All doses of DL (25-400 mg/kg) produced less anti-inflammatory effect than IND (Table 42).

Table 37 Change of edema volume (ml) of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

Treatments (mg/kg)	Paw edema (ml)±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2% Tween 80	0.029±0.0051	0.0525±0.0031	0.0913±0.0058	0.0775±0.0082	0.0675±0.0118	0.0400±0.0091
IND 10 mg/kg	0.0250±0.0019 (-13.79%)	0.0313±0.0029* (-40.48%)	0.0400±0.0060*** (-56.19%)	0.0238±0.0037*** (-69.35%)	0.0188±0.0023** (-72.22%)	0.0338±0.0053 (-15.63%)
BMY 125 mg/kg	0.0338±0.0046 (16.38%)	0.0500±0.0063 (-4.76%)	0.0613±0.0069* (-32.91%)	0.0675±0.0053 (-12.90%)	0.0750±0.0082 (11.11%)	0.0550±0.0057 (37.50%)
BMY 250 mg/kg	0.0338±0.0053 (16.38%)	0.0575±0.0070 (9.52%)	0.0463±0.0080*** (-49.34%)	0.0375±0.0049*** (-51.61%)	0.0400±0.0073 (-40.74%)	0.0463±0.0073 (15.63%)
BMY 500 mg/kg	0.0375±0.0031 (29.31%)	0.0513±0.0040 (-2.38%)	0.0313±0.0040*** (-65.77%)	0.0338±0.0056*** (-56.45%)	0.0338±0.0068* (-50%)	0.0325±0.0041 (-18.75%)

Table 38 Change of edema volume (ml) of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and *Aegle marmelos* root extract (AM; 25-400 mg/kg). N=8. $^{*}p<0.05$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

Treatments (mg/kg)	Paw edema±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2% Tween 80	0.0513±0.0048	0.0625±0.0041	0.0750±0.0046	0.0838±0.0037	0.0850±0.0073	0.0788±0.0058
IND 10 mg/kg	0.0400±0.0019 (-22.03%)	0.0350±0.0019*** (-44%)	0.0275±0.0025*** (-63.33%)	0.0275±0.0025*** (-67.18%)	0.0313±0.0035*** (-63.24%)	0.0375±0.0037*** (-52.41%)
AM 25 mg/kg	0.0463±0.0032 (-9.84%)	0.0625±0.0031 (0%)	0.0738±0.0026 (-1.67%)	0.0825±0.0049 (-1.55%)	0.0800±0.0066 (-5.88%)	0.0788±0.0061 (-0.06%)
AM 50 mg/kg	0.0438±0.0018 (-14.72%)	0.0600±0.0033 (-4.00%)	0.0725±0.0059 (-3.33%)	0.0800±0.0053 (-4.53%)	0.0775±0.0037 (-8.82%)	0.0763±0.0042 (-3.24%)
AM 100 mg/kg	0.0413±0.0029 (-19.59%)	0.0575±0.0041 (-8.00%)	0.0700±0.0038 (-6.67%)	0.0763±0.0050 (-9.01%)	0.0738±0.0073 (-13.24%)	0.0750±0.0089 (-4.82%)
AM 200 mg/kg	0.0413±0.0035 (-19.59%)	0.0550±0.0042 (-12.00%)	0.0650±0.0038 (-13.33%)	0.0713±0.0035 (-14.98%)	0.0700±0.0033 (-17.65%)	0.0663±0.0046 (-15.93%)
AM 400 mg/kg	0.0413±0.0023 (-19.59%)	0.0525±0.0031 (-16.00%)	0.0575±0.0016* (-23.33%)	0.0638±0.0026* (-23.93%)	0.0625±0.0059 (-26.47%)	0.0625±0.0067 (-20.69%)

Table 39 Change of edema (ml) volume of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and *Dimocarpus longan* root extract (DL; 25-400 mg/kg). N=8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

Treatments (mg/kg)	Paw edema±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2% Tween 80	0.0425±0.0049	0.0725±0.0077	0.0850±0.0089	0.0825±0.0065	0.0725±0.0092	0.0613±0.0079
IND 10 mg/kg	0.0313±0.0012 (-26.47%)	0.0300±0.0038** (-58.62%)	0.0263±0.0026*** (-69.12%)	0.0238±0.0026*** (-71.21%)	0.0300±0.0042*** (-58.62%)	0.0363±0.0046 (-40.86%)
DL 25 mg/kg	0.0438±0.0026 (2.94%)	0.0725±0.0053 (0%)	0.0825±0.0065 (-2.94%)	0.0788±0.0072 (-4.55%)	0.0713±0.0051 (-1.72%)	0.0600±0.0087 (-2.12%)
DL 50 mg/kg	0.0438±0.0026 (2.94%)	0.0713±0.00581 (-1.72%)	0.0738±0.0073 (-13.24%)	0.0713±0.0064 (-13.64%)	0.0650±0.0053 (-10.34%)	0.0575±0.0075 (-6.20%)
DL 100 mg/kg	0.0413±0.0035 (-2.94%)	0.0688±0.0029 (-5.17%)	0.0700±0.0046 (-17.65%)	0.0688±0.0058 (-16.67%)	0.0575±0.0041 (-20.69%)	0.0500±0.0087 (-18.43%)
DL 200 mg/kg	0.0400±0.0042 (-5.88%)	0.0638±0.0125 (-12.07%)	0.0575±0.0073* (-32.35%)	0.0538±0.0068* (-34.85%)	0.0488±0.0040* (-32.76%)	0.0475±0.0025 (-22.51%)
DL 400 mg/kg	0.0388±0.0029 (-8.82%)	0.0625±0.0037 (-13.79%)	0.0538±0.0037* (-36.76%)	0.0500±0.0033** (-39.39%)	0.0463±0.0037* (-36.21%)	0.0425±0.0077 (-30.67%)

Table 40 Change of edema volume (ml) of oral administration of 2% Tween 80, indomethacin (IND 10 mg/kg) and *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). N=8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

Treatments (mg/kg)	Paw edema±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2% Tween 80	0.0463±0.0026	0.0575±0.0036	0.0725±0.0049	0.0800±0.0057	0.0738±0.0053	0.0688±0.0048
IND 10 mg/kg	0.0388±0.0029 (-16.13%)	0.0388±0.0035** (-32.61%)	0.0300±0.0038*** (-58.62%)	0.0288±0.0029*** (-64.06%)	0.0288±0.0029*** (-60.99%)	0.0325±0.0031** (-52.69%)
DS 25 mg/kg	0.0450±0.0019 (-2.60%)	0.0550±0.0027 (-4.35%)	0.0700±0.0046 (-3.45%)	0.0688±0.0079 (-14.06%)	0.0688±0.0079 (-6.72%)	0.0688±0.0087 (0.07%)
DS 50 mg/kg	0.0438±0.0026 (-5.30%)	0.0538±0.0026 (-6.52%)	0.0688±0.0035 (-5.17%)	0.0675±0.0036 (-15.63%)	0.0675±0.0036 (-8.41%)	0.0663±0.0050 (-3.57%)
DS 100 mg/kg	0.0438±0.0026 (-5.30%)	0.0525±0.0025 (-8.70%)	0.0650±0.0038 (-10.34%)	0.0638±0.0042 (-20.31%)	0.0638±0.0042 (-13.50%)	0.0625±0.0045 (-9.02%)
DS 200 mg/kg	0.0425±0.0016 (-8.01%)	0.0525±0.0016 (-8.70%)	0.0588±0.0029 (-18.97%)	0.0563±0.0062* (-29.69%)	0.0575±0.0065 (-21.98%)	0.0575±0.0059 (-16.30%)
DS 400 mg/kg	0.0413±0.0012 (-10.71%)	0.0513±0.0035 (-10.87%)	0.0538±0.0046* (-25.86%)	0.0525±0.0053* (-34.38%)	0.0538±0.0056 (-27.07%)	0.0538±0.0059 (-21.76%)

Table 41 Change of edema volume (ml) of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and *Oroxylum indicum* root extract (OI; 25-400 mg/kg). N=8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

Treatments (mg/kg)	Paw edema±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2% Tween 80	0.0488±0.0035	0.0600±0.0033	0.0700±0.0027	0.0763±0.0026	0.0738±0.0053	0.0700±0.0065
IND 10 mg/kg	0.0375±0.0025 (-23%)	0.0275±0.0016*** (-54.17%)	0.0225±0.0016*** (-67.86%)	0.0238±0.0018*** (-68.83%)	0.0300±0.0033*** (-59.29%)	0.0338±0.0026*** (-51.79%)
OI 25 mg/kg	0.0488±0.0035 (0.10%)	0.0613±0.0044 (2.08%)	0.0700±0.0042 (0%)	0.0738±0.0026 (-3.22%)	0.0738±0.0042 (0.07%)	0.0725±0.0059 (3.57%)
OI 50 mg/kg	0.0475±0.0053 (-2.46%)	0.0600±0.0063 (0%)	0.0675±0.0062 (-3.57%)	0.0725±0.0070 (-4.86%)	0.0713±0.0058 (-3.32%)	0.0700±0.0053 (0%)
OI 100 mg/kg	0.0475±0.0041 (-2.46%)	0.0575±0.0025 (-4.17%)	0.0650±0.0053 (-7.14%)	0.0713±0.0044 (-6.50%)	0.0688±0.0061 (-6.72%)	0.0688±0.0051 (-1.79%)
OI 200 mg/kg	0.0450±0.0033 (-7.60%)	0.0500±0.0042 (-16.67%)	0.0525±0.0045* (-25%)	0.0563±0.0037* (-26.18%)	0.0588±0.0055 (-20.28%)	0.0613±0.0061 (-12.50%)
OI 400 mg/kg	0.0425±0.0025 (-12.73%)	0.0463±0.0032 (-22.92%)	0.0475±0.0041* (-32.14%)	0.0525±0.0056** (-31.10%)	0.0538±0.0050* (-27.07%)	0.0575±0.0056 (-17.86%)

Table 42 Change of edema volume (ml) of oral administration of 2%Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and *Walsura trichostemon* root extract (WT; 25-400 mg/kg). N=8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2%Tween 80.

Treatments (mg/kg)	Paw edema±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2%Tween 80	0.055±0.0042	0.0675±0.0036	0.085±0.0060	0.0863±0.0050	0.0763±0.0042	0.0675±0.0036
IND 10 mg/kg	0.0438±0.0032 (-20.45%)	0.0325±0.0025*** (-51.85%)	0.0225±0.0016*** (-73.53%)	0.0238±0.0018*** (-72.45%)	0.0288±0.0029*** (-62.27%)	0.0325±0.0041** (-51.85%)
WT 25 mg/kg	0.0500±0.0075 (-9.09%)	0.0525±0.0075 (-22.22%)	0.0538±0.0086** (-36.76%)	0.0588±0.0089* (-31.84%)	0.0688±0.0074 (-9.78%)	0.0700±0.0084 (-3.70%)
WT 50 mg/kg	0.0513±0.0055 (-6.82%)	0.0525±0.0049 (-22.22%)	0.0488±0.0051** (-42.65%)	0.0563±0.0046** (-34.74%)	0.0663±0.0046 (-13.06%)	0.0688±0.0048 (1.85%)
WT 100 mg/kg	0.0488±0.0040 (-11.36%)	0.0500±0.0038 (-25.93%)	0.0488±0.0074** (-42.65%)	0.0563±0.0075** (-34.74%)	0.0638±0.0080 (-16.34%)	0.0675±0.0084 (0%)
WT 200 mg/kg	0.0475±0.0041 (-13.64%)	0.0500±0.0033 (-25.93%)	0.0450±0.0033*** (-47.06%)	0.0500±0.0042** (-42%)	0.0575±0.0041 (-24.54%)	0.0613±0.0051 (-9.26%)
WT 400 mg/kg	0.0475±0.0031 (-13.64%)	0.0475±0.0049 (-29.63%)	0.0438±0.0046*** (-48.53%)	0.0463±0.0042*** (-46.35%)	0.0525±0.0025* (-31.10%)	0.0563±0.0032 (-16.67%)

Anti-nociceptive activity

Mouse hot-plate test

To demonstrate the validity of the hot-plate analgesic testing following drug administration, mice received morphine sulphate (MO; 10 mg/kg) intraperitoneally (i.p.) and were tested during the subsequent 240 min period. As expected MO significantly ($p<0.001$) increased the hot-plate latency producing an area of analgesia of 12,835.65±1,909.33 %MPE-min compared with that of normal saline solution (NSS) (-10,873.10±4,166.42 %MPE-min; Figure 68).

Initial studies utilizing the hot-plate test in mice to examine the efficacy of Ben-Cha-Moon-Yai remedy and five root species extracts in producing analgesia. Mice were administered orally 2% Tween 80 or various doses of Ben-Cha-Moon-Yai remedy (BMY; 125, 250 and 500 mg/kg) and five root species extracts (25, 50, 100, 200 and 400 mg/kg).

All doses of BMY (125, 250 and 500 mg/kg) significantly ($p<0.05$, $p<0.001$ and $p<0.01$, respectively) increased the hot-plate latency when compared to the vehicle group. BMY 250 mg/kg significantly ($p<0.05$) increased the hot-plate latency when compared to BMY 125 mg/kg (Figure 69). The analgesic peak effects of BMY 125, 250 and 500 mg/kg were reached within 90, 240, 120 min after oral administration, respectively.

AM 400 mg/kg significantly ($p<0.05$) increased the hot-plate latency when compared to the vehicle group (Figure 70). The analgesic peak effect of AM 400 mg/kg was reached within 120 min after oral administration.

DS at the doses of 200 and 400 mg/kg significantly ($p<0.01$, $p<0.05$, respectively) increased the hot-plate latency when compared to the vehicle group (Figure 70). The analgesic peak effects of DS 200 and 400 mg/kg were reached within 120 and 90 min after oral administration, respectively.

All doses of DL and OI (25-400 mg/kg) did not produce analgesic response when compared to the vehicle group (Figure 71 and 73).

WT at the doses of 100, 200 and 400 mg/kg significantly ($p<0.05$, $p<0.01$ and $p<0.01$, respectively) increased the hot-plate latency when compared to the vehicle group (Figure 74). The analgesic peak effects of WT 100, 200 and 400 mg/kg were reached within 120, 30 and 90 min after oral administration, respectively.

In order to investigate any role of the opioid receptor in AM, DS and WT actions, mice were then administered NSS (10 ml/kg, i.p.), naloxone (NAL; 5 mg/kg, i.p.), a short-acting opioid receptor antagonist, 2% Tween 80 (10 ml/kg, p.o.), AM (400 mg/kg, p.o.), DS (200 mg/kg, p.o.), WT (400 mg/kg, p.o.) or the combination of NAL and AM (5/400 mg/kg), the combination of NAL and DS (5/200 mg/kg) and the combination of NAL and WT (5/400 mg/kg). NAL alone failed to produce significant response when compared to vehicle group. AM, DS, WT at the dose tested produced significant ($p<0.001$) response when compared to vehicle group. The inclusion of naloxone with AM, DS and WT significantly ($p<0.001$) attenuated the analgesic response due to AM, DS and WT indicating that opioid receptors are involved in the analgesic response produced by AM, DS and WT (Figure 75, 76, 77, respectively).

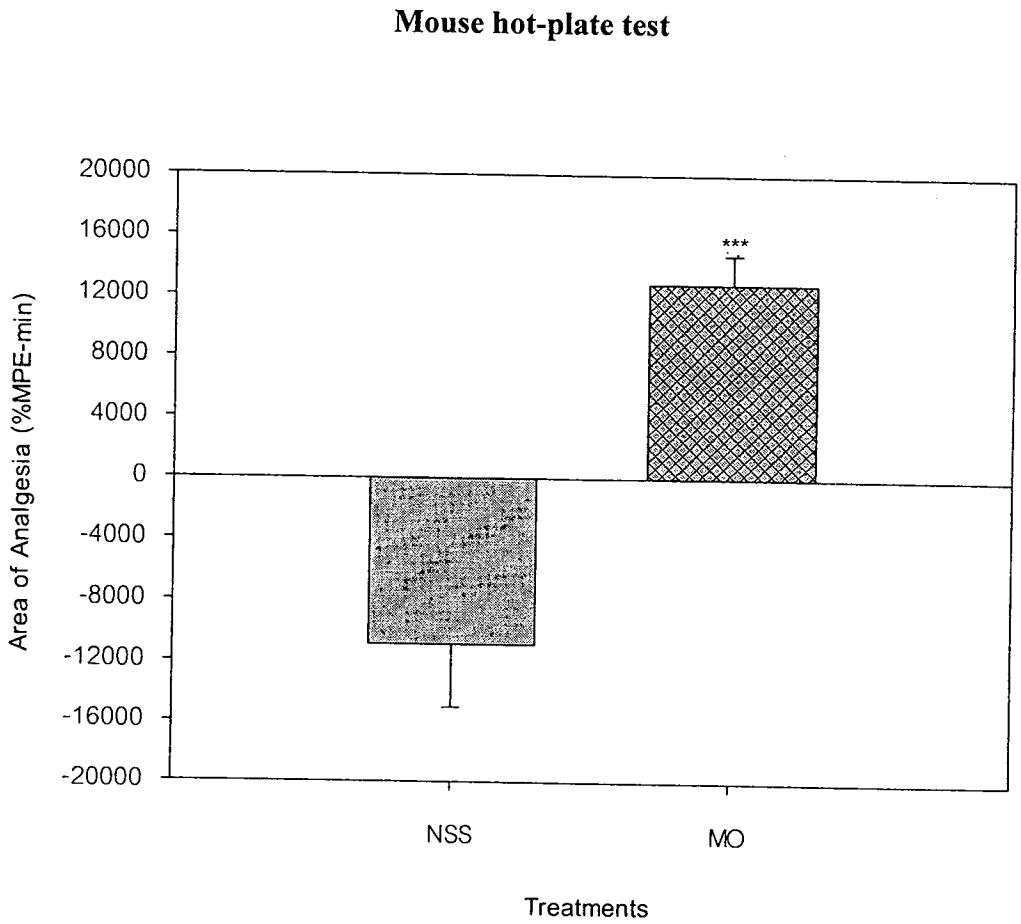


Figure 68 Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of normal saline solution (NSS; 10 ml/kg) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. $^{***}p<0.001$ significantly different compared to NSS.

Mouse hot-plate test

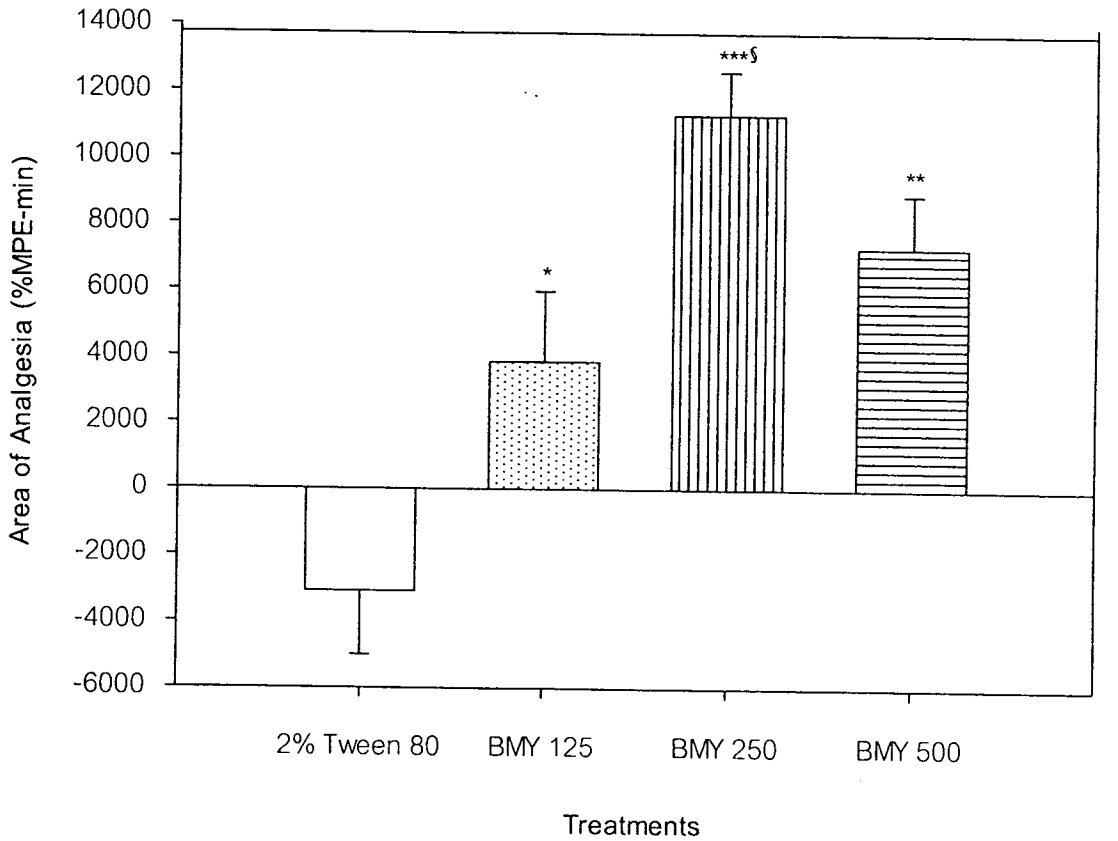


Figure 69 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 (10 ml/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=10 for all groups.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$ significantly different compared to BMY 125 mg/kg.

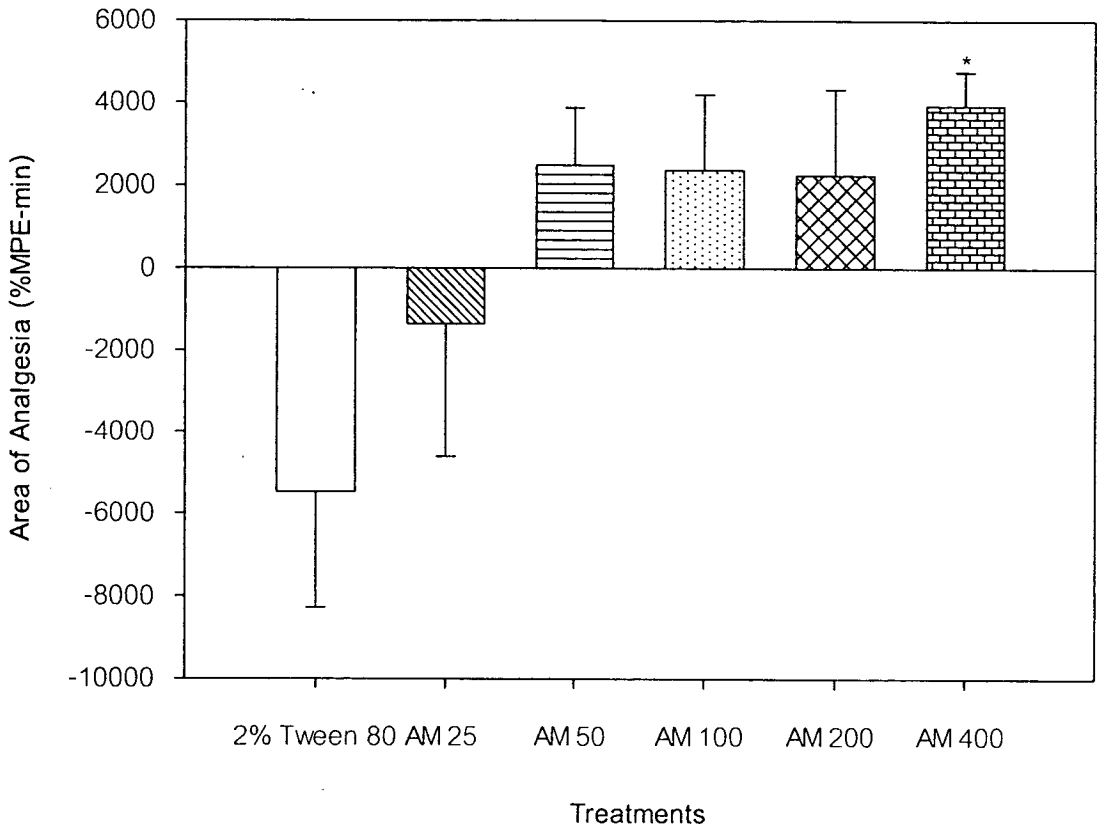


Figure 70 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Aegle marmelos* root extract (AM; 25-400 mg/kg). N=10 for all groups. $^{*}p<0.05$ significantly different compared to 2% Tween 80.

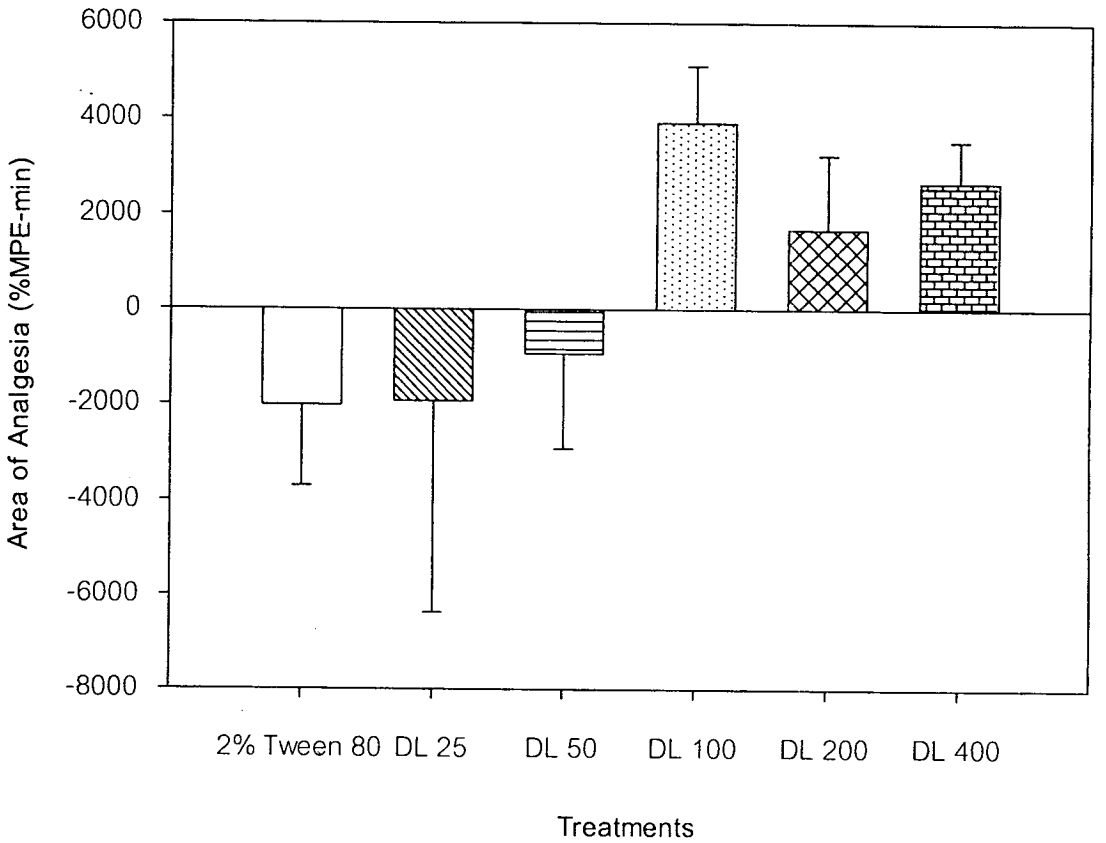


Figure 71 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg). N=10 for all groups.

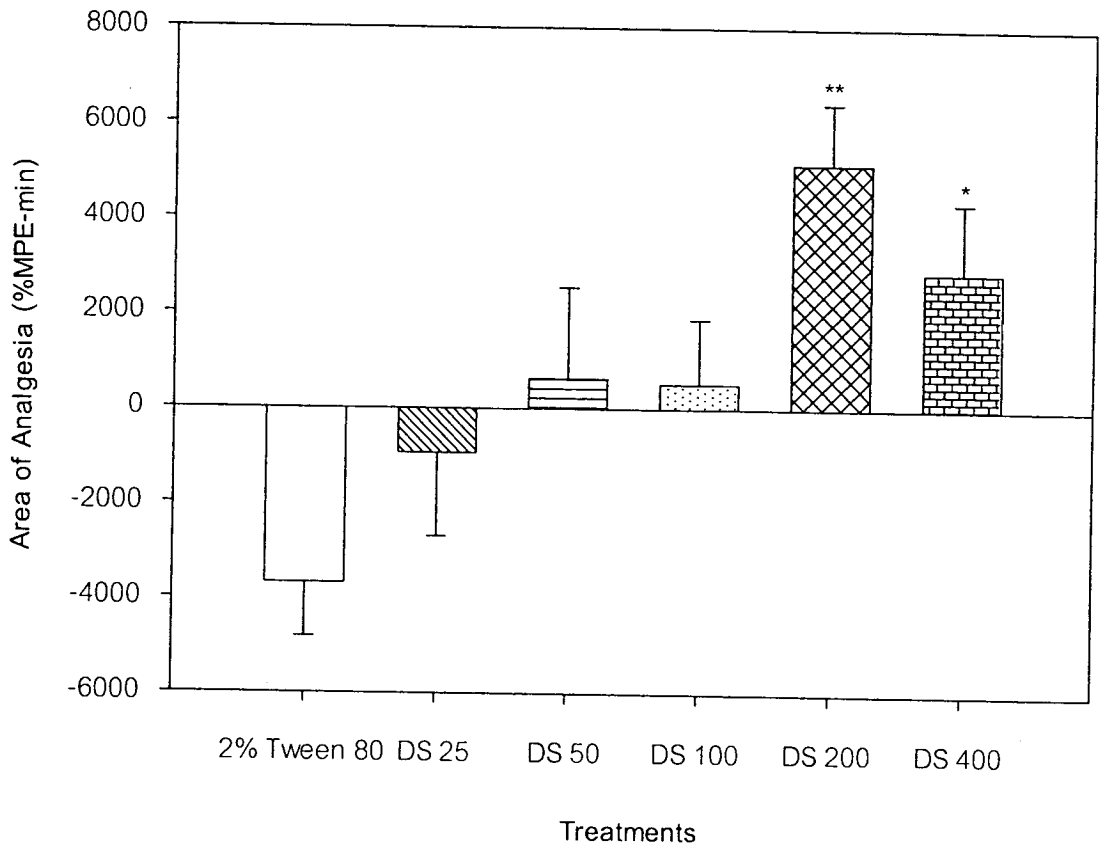


Figure 72 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). N=10 for all groups. $^{*}p<0.05$, $^{**}p<0.01$ significantly different compared to 2% Tween 80.

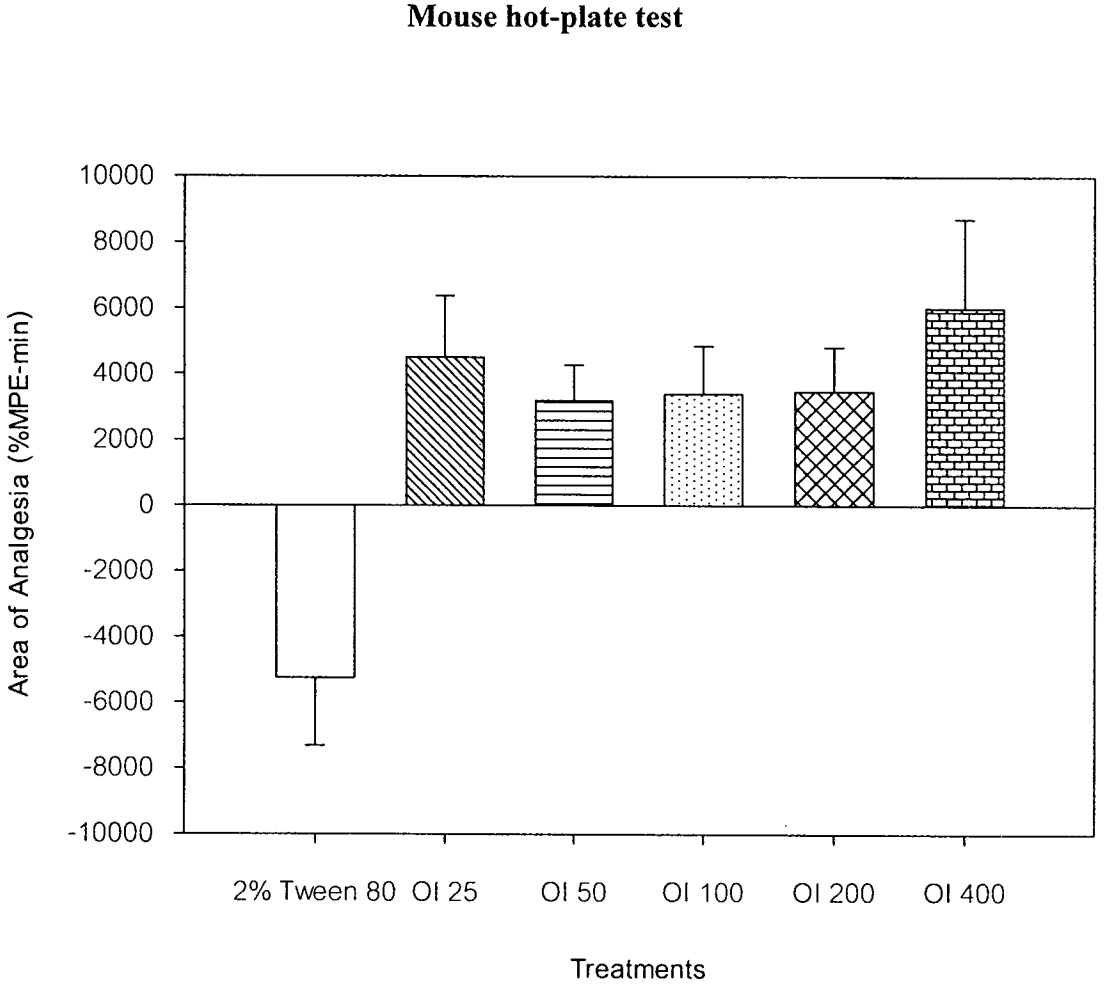


Figure 73 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg). N=10 for all groups.

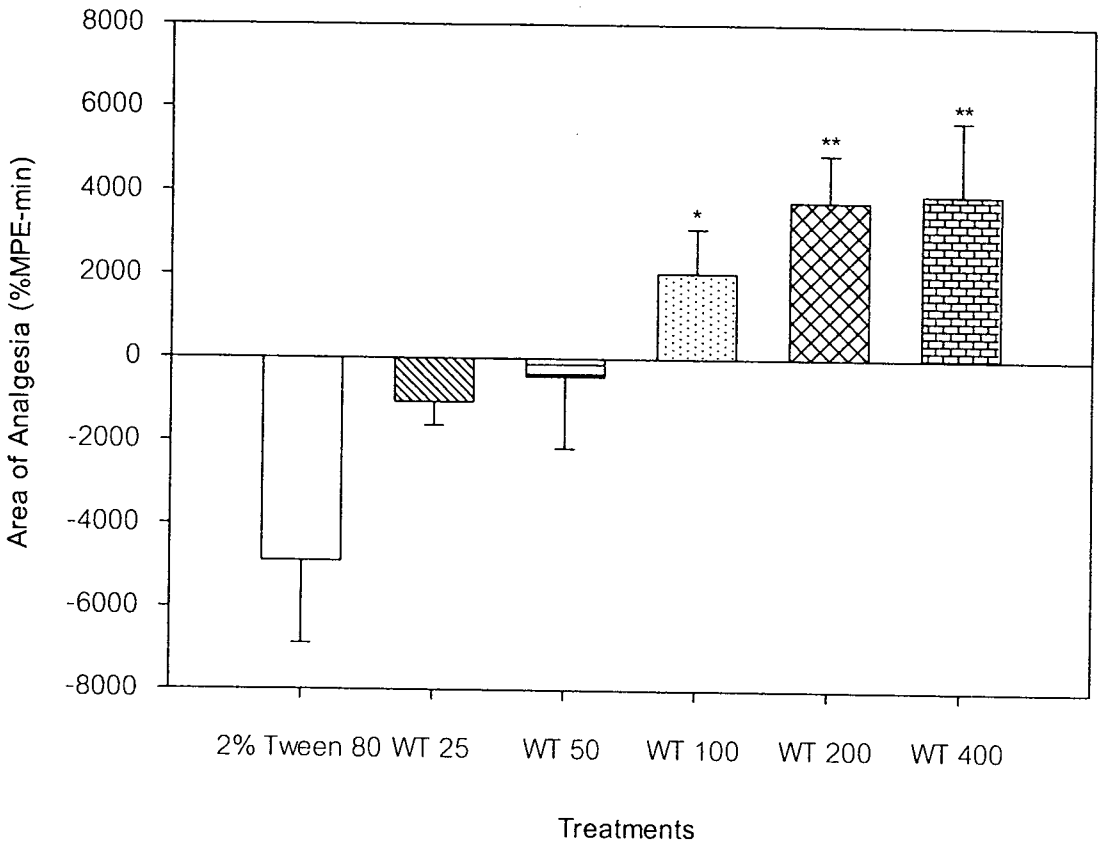


Figure 74 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Walsura trichostemon* root extract (WT; 25-400 mg/kg). N=10 for all groups. $^{*}p<0.05$, $^{**}p<0.01$ significantly different compared to 2% Tween 80.

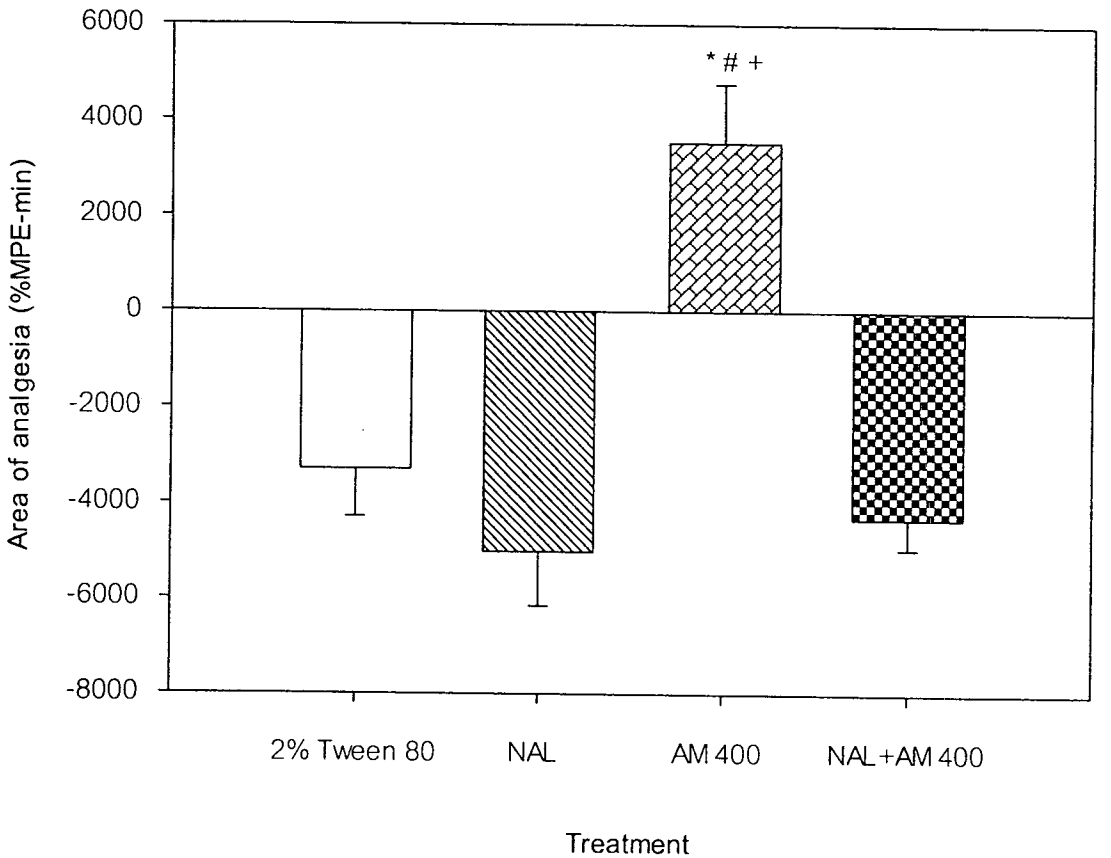


Figure 75 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), *Aegle marmelos* root extract (AM; 400 mg/kg, p.o.) and the combination of naloxone and AM (5/400 mg/kg). N=10 for all groups.

$^{*}p<0.001$ significantly different compared to 2% Tween 80.

$^{\#}p<0.001$ significantly different compared to NAL.

$^{+}p<0.001$ significantly different compared to NAL+AM 400.

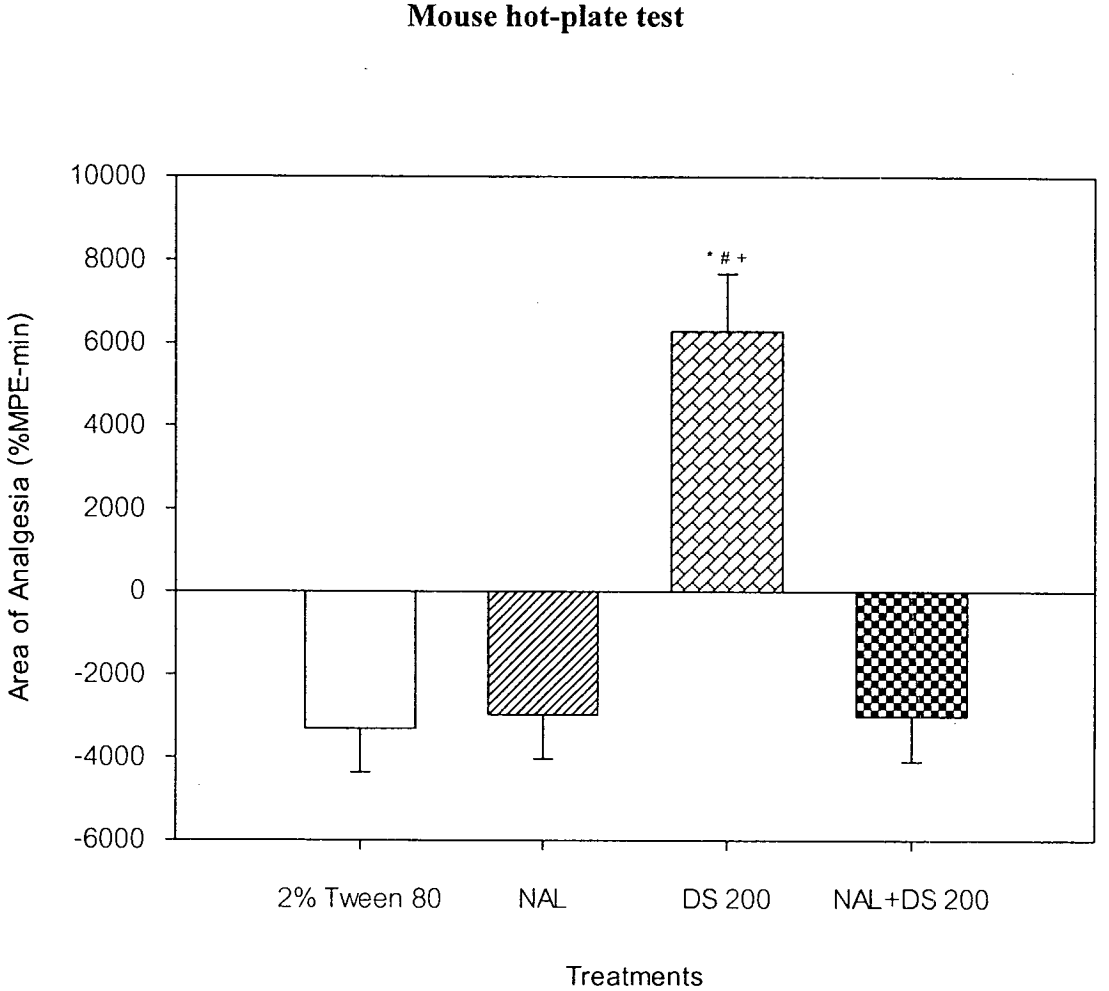


Figure 76 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), *Dolichandrone serrulata* root extract (DS; 200 mg/kg, p.o.) and the combination of naloxone and DS (5/200 mg/kg). N=10 for all groups.

$^{*}p<0.001$ significantly different compared to 2% Tween 80.

$^{\#}p<0.001$ significantly different compared to NAL.

$^{+}p<0.001$ significantly different compared to NAL+DS 200.

Mouse hot-plate test

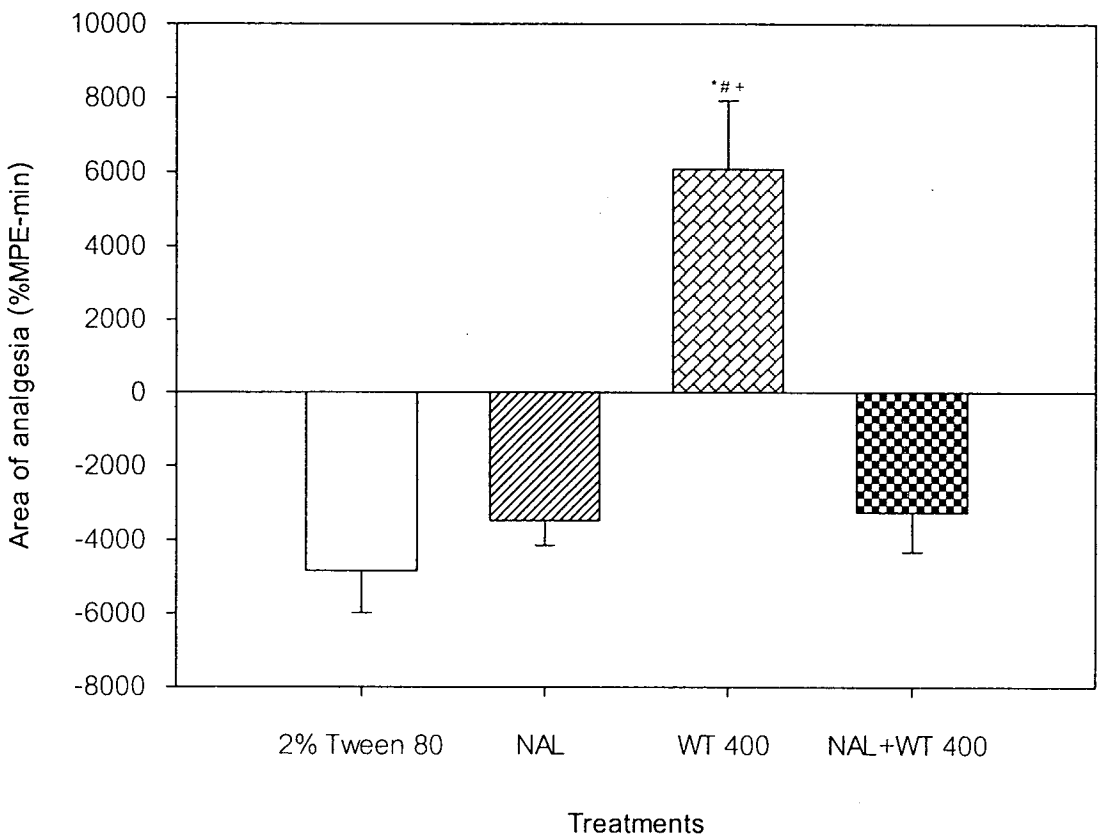


Figure 77 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), *Walsura trichostemon* root extract (WT; 400 mg/kg, p.o.) and the combination of naloxone and WT (5/400 mg/kg). N=10 for all groups.

$^{*}p<0.001$ significantly different compared to 2% Tween 80.

$^{\#}p<0.001$ significantly different compared to NAL.

$^{+}p<0.001$ significantly different compared to NAL+WT 400.

Formalin test in mice

To demonstrate the validity of formalin test following drug administration, mice received morphine sulfate (MO; 10 mg/kg) intraperitoneally or indomethacin (IND; 10 mg/kg) and were observed for paw licking at early phase (0-5 min) and late phase (15-30 min). As expected MO significantly ($p<0.001$ and $p<0.001$, respectively) decreased the licking time of both early and late phases by 90.87% and 97.69%, respectively producing mean time spent on paw licking of 7.48±1.84 and 3.67±1.08 sec compared with that of NSS (81.92±8.60 and 158.80±6.08 sec, respectively; Figure 78, Table 43).

Study then utilized the formalin test in mice to examine the efficacy of BMY and five herbal root extracts (AM, OI, DL, DS and WT) in producing analgesia. Mice were administered orally 2% Tween 80, IND (10 mg/kg), various doses of BMY (125, 250 and 500 mg/kg) or AM, OI, DL, DS and WT (25, 50, 100, 200 and 400 mg/kg).

Only BMY 250 mg/kg significantly ($p<0.05$) decreased the licking time by 20.03% producing mean time spent on paw licking of 90.82±6.93 sec compared with that of vehicle group (113.56±4.83 sec) in the early phase. For the late phase, All doses of BMY (125, 250 and 500 mg/kg) significantly ($p<0.01$) decreased the licking time by 48.96%, 56.85% and 51.30%, respectively producing mean time spent on paw licking of 80.97±18.20, 68.47±14.99 and 77.27±18.43 sec, respectively when compared with that of vehicle group (158.66±13.07 sec). The reference drug, IND (10 mg/kg) also caused significant ($p<0.01$) inhibition of the late phase of formalin-induced nociception, producing 56.58% inhibition when compared to the vehicle group (Figure 79, Table 44).

AM at dose of 400 mg/kg significantly ($p<0.05$) decreased the licking time in the early phase by 34.55% producing mean time spent on paw licking of 64.19±4.08 sec compared with that of vehicle group (98.06±5.45 sec). Furthermore, AM 400 mg/kg also significantly ($p<0.01$ and $p<0.05$, respectively) decreased the licking time in the early phase compared with that of AM 25 and 100 mg/kg (103.42±6.64 and 98.60±4.03 sec, respectively). IND (10 mg/kg), the reference drug, caused significant

(p<0.001) inhibition of the late phase of formalin-induced nociception, producing 61.71% inhibition when compared to the vehicle group. AM 400 mg/kg significantly (p<0.001) decreased the licking time by and 59.85% producing the mean time spent on paw licking of 58.44±5.50 sec compared with that of vehicle group (145.57±17.16 sec). The antinociceptive efficacy of AM 400 mg/kg is comparable to IND. Additionally, AM 400 mg/kg significantly (p<0.001, p<0.001, p<0.001 and p<0.01, respectively) decreased the licking time in the late phase compared with that of AM 25, 50, 100 and 200 mg/kg (Figure 80, Table 45).

All doses of OI failed to decrease the licking time in the early phase induced by formalin. However, OI at the doses of 100, 200 and 400 mg/kg significantly (p<0.01, p<0.001, p<0.001, respectively) decreased the licking time by 49.40%, 61.78% and 68.28%, respectively producing mean time spent on paw licking of 73.07±9.98, 55.18±9.03 and 45.80±17.08 sec, respectively compared with that of vehicle group (144.41±10.68 sec) during the late phase. IND (10 mg/kg) caused significant (p<0.01) inhibition of the late phase of formalin-induced nociception, producing 48.96% inhibition when compared to the vehicle group. In addition, OI 100 mg/kg significantly (p<0.05) decreased the licking time in the late phase compared with that of OI 25 mg/kg. OI 200 mg/kg significantly (p<0.001 and p<0.01, respectively) decreased the licking time in the late phase compared with that of OI 25 and 50 mg/kg. OI 400 mg/kg significantly (p<0.001) decreased the licking time in the late phase compared with that of OI 25 and 50 mg/kg. The antinociceptive efficacy during the late phase of OI 50-400 mg/kg is comparable to IND (Figure 81, Table 46).

All doses of DL failed to decrease the licking time in the early phase induced by formalin. However, DL 200 and 400 mg/kg significantly (p<0.01 and p<0.001, respectively) decreased the licking time during the late phase by 53.57% and 88.14%, respectively producing mean time spent on paw licking of 56.90±12.12 and 14.52±6.54 sec, respectively compared with that of vehicle group (122.54±15.26 sec). IND (10 mg/kg) caused significant (p<0.01) inhibition only in the late phase of formalin-induced nociception, producing 56.39% inhibition when compared to the vehicle group. Furthermore, in the late phase, DL 200 mg/kg significantly (p<0.01 and p<0.05, respectively) decreased the licking time compared with that of DL 25 and

50 mg/kg. DL 400 mg/kg also significantly ($p<0.001$) decreased the licking time in the late phase compared with that of DL 25-100 mg/kg. The antinociceptive efficacy during the late phase of DL 200 and 400 mg/kg are comparable to IND (Figure 82, Table 47).

DS 400 mg/kg significantly ($p<0.001$) decreased the licking time in the early phase by 38.17% producing mean time spent on paw licking of 64.53±3.99 sec compared with that of vehicle group (104.38±5.50 sec). DS 400 mg also significantly ($p<0.01$, $p<0.05$, $p<0.01$ and $p<0.05$, respectively) decreased mean time spent on paw licking in the early phase compared with that of IND and DS at the doses of 25, 50 and 100 mg/kg, respectively. For the late phase, DS 200 and 400 mg/kg significantly ($p<0.01$, $p<0.001$, respectively) decreased the licking time in the late phase by 45.22% and 55.31%, respectively producing mean time spent on paw licking of 73.99±7.81 and 60.36±5.32 sec, respectively compared with that of vehicle group (135.07±10.01 sec). DS 200 mg/kg significantly ($p<0.01$) decreased the licking time in the late phase compared with that of DS 25 mg/kg. DS 400 mg/kg significantly ($p<0.001$) decreased the licking time compared with that of DS 25 mg/kg. IND (10 mg/kg) caused significant ($p<0.001$) inhibition of the late phase of formalin-induced nociception, producing 61.15% inhibition when compared to the vehicle group. The antinociceptive efficacy during the late phase of DS 200 and 400 mg/kg are comparable to IND (Figure 83, Table 48).

In the early phase, WT 400 mg kg significantly ($p<0.05$) decreased the licking time by 31.66% producing mean time spent on paw licking of 72.32±4.15 sec compared with that of vehicle group (105.03±6.11 sec). WT 400 mg/kg significantly ($p<0.01$ and $p<0.05$, respectively) decreased the licking time compared with that of WT 50 and 100 mg/kg. For the late phase, WT 200 and 400 mg/kg significantly ($p<0.01$ and $p<0.001$, respectively) decreased the licking time by 44.49% and 53% producing mean time spent on paw licking of 78.59±7.71 and 66.55±5.82 sec, respectively compared with that of vehicle group (141.59±13.19 sec). WT 400 mg/kg also significantly ($p<0.01$) decreased the licking time compared with that of WT 25 mg/kg. IND (10 mg/kg) caused significant ($p<0.001$) inhibition of the late phase of formalin-induced nociception, producing 61.74% inhibition when compared to the

vehicle group. The antinociceptive efficacy during the late phase of WT 200 and 400 mg/kg are comparable to IND (Figure 84, Table 49).

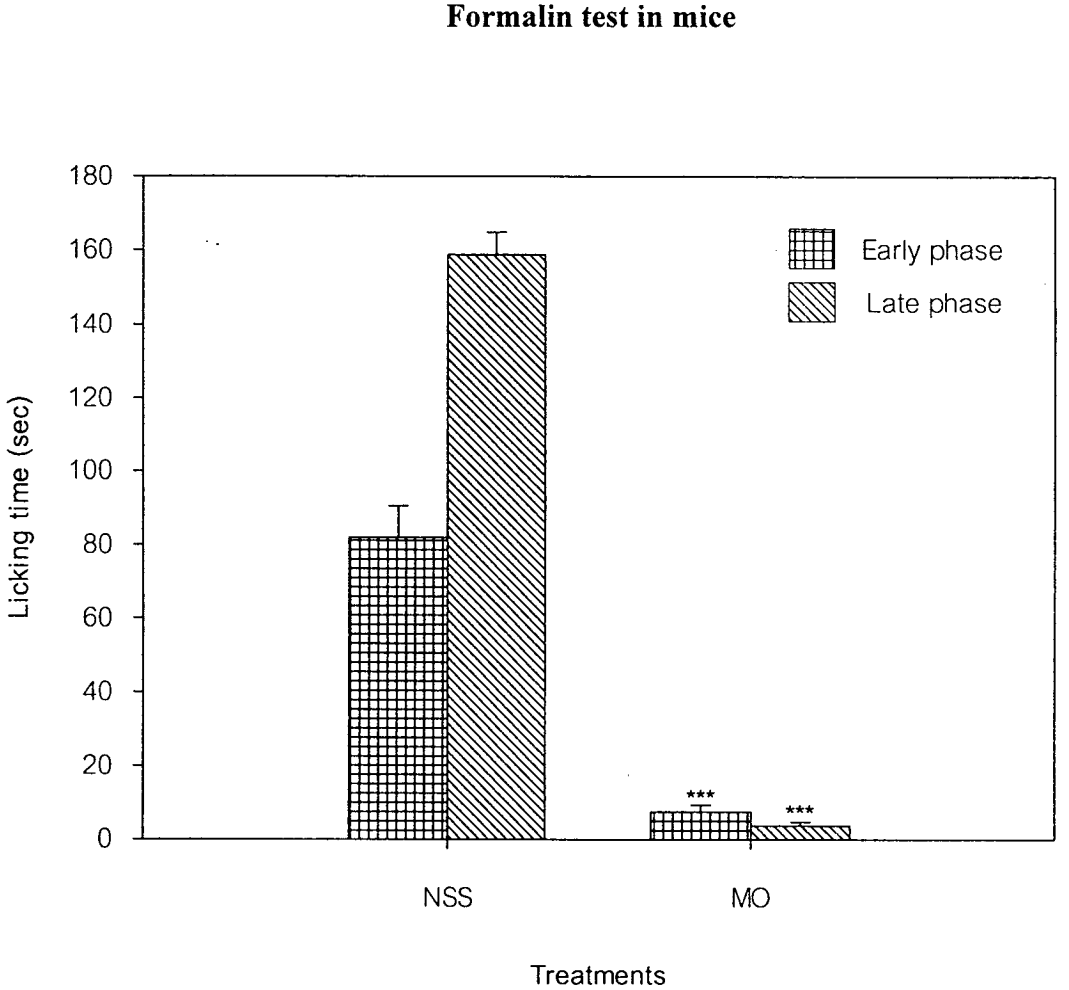


Figure 78 Time spent on paw licking after intraperitoneal administration of 0.9% normal saline solution (NSS; 10 ml/kg) and morphine sulphate (MO; 10 mg/kg). N=8 for all groups. $^{***}p<0.001$ significantly different compared to NSS.

Formalin test in mice

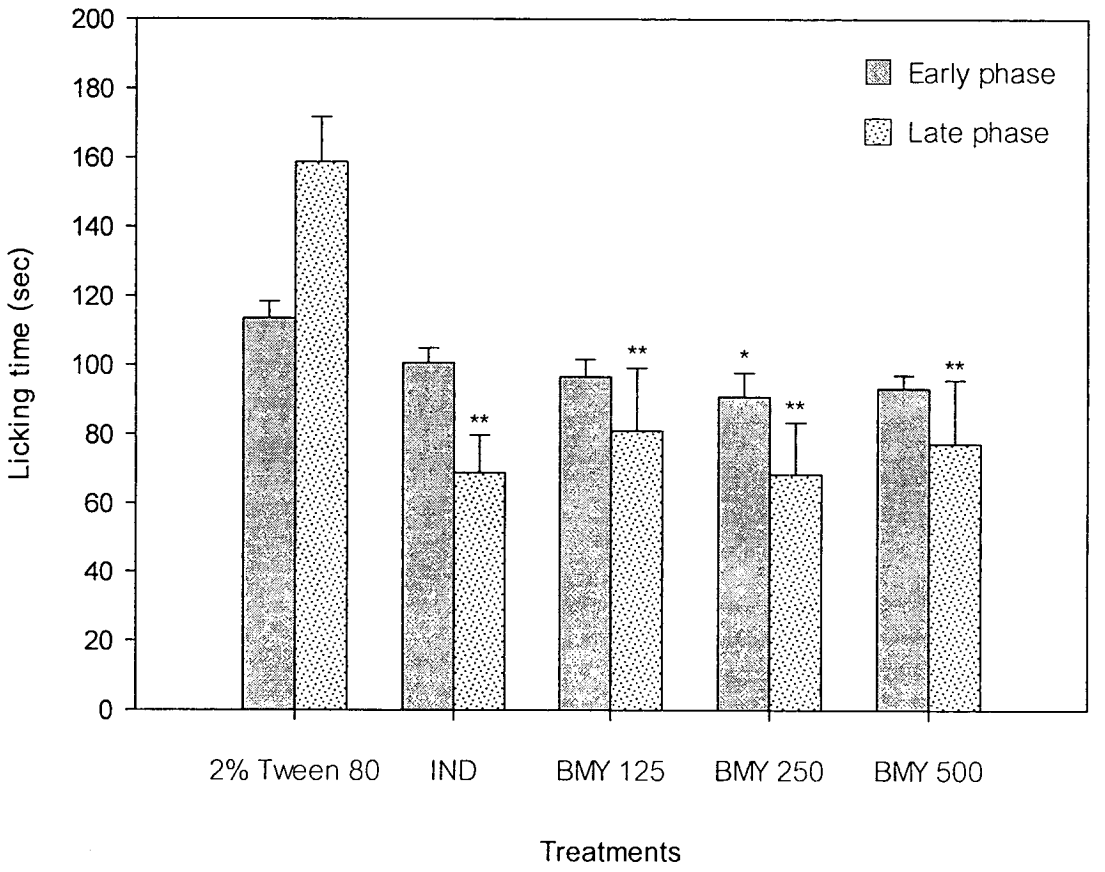


Figure 79 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=8 for all groups. $^{*}p<0.05$, $^{**}p<0.01$ significantly different compared to 2% Tween 80.

Formalin test in mice

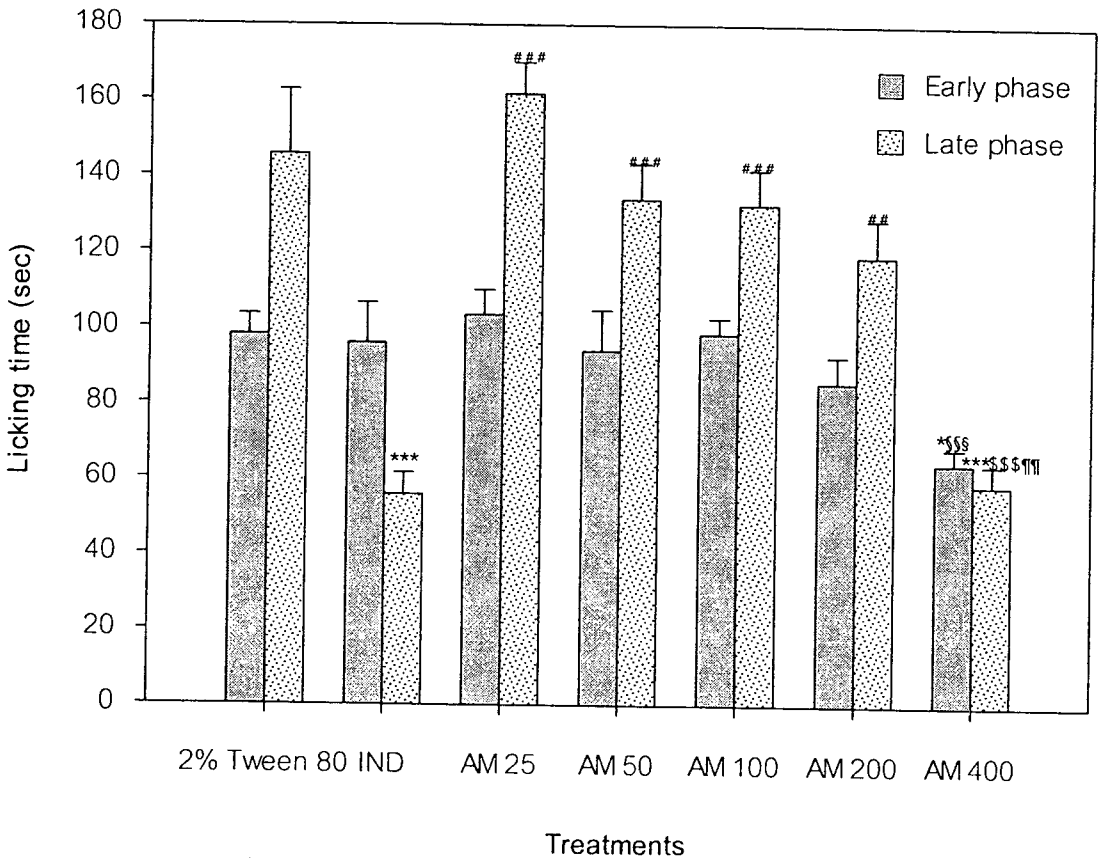


Figure 80 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Aegle marmelos* root extract (AM; 25-400 mg/kg). N=8 for all groups.

$^{*}p<0.05$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$ significantly different compared to AM 100 mg/kg.

$^{\P\P}p<0.01$ significantly different compared to AM 200 mg/kg.

$^{\S\S}p<0.01$ significantly different compared to AM 25 mg/kg.

$^{\$\$\$}p<0.001$ significantly different compared to AM 25-100 mg/kg.

$^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Formalin test in mice

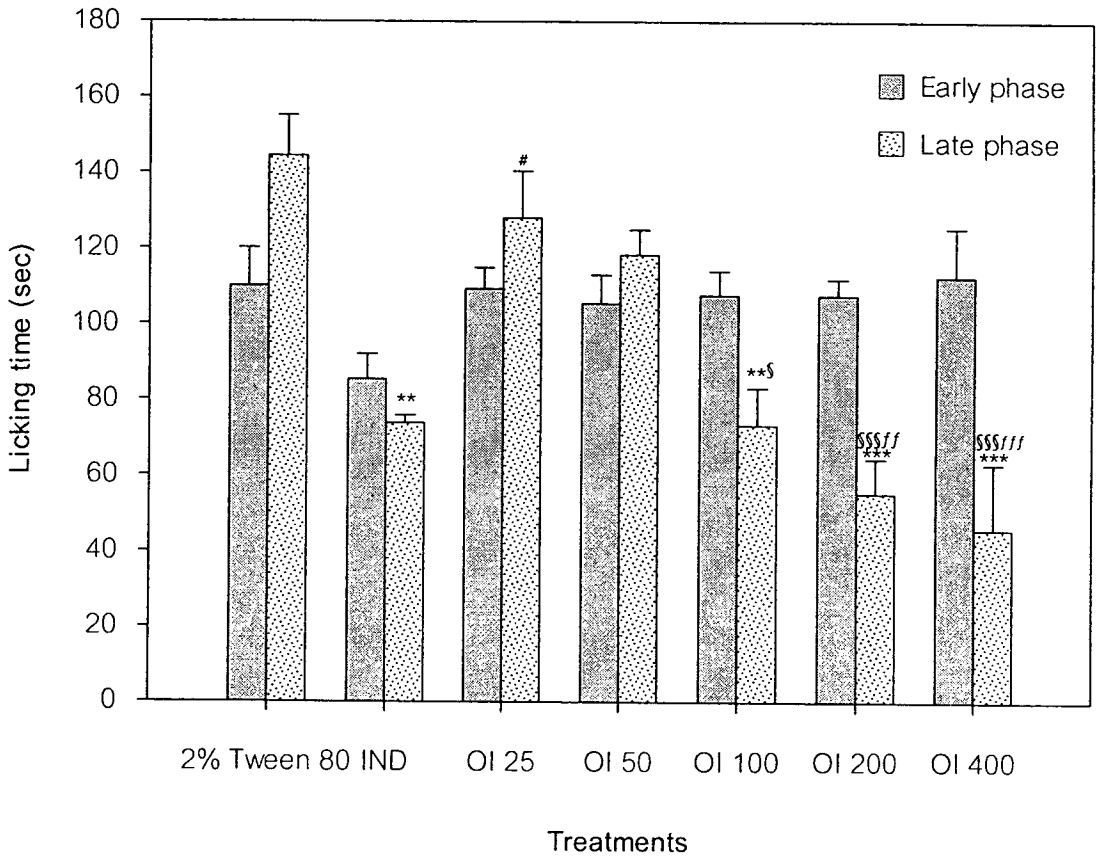


Figure 81 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg). N=8 for all groups.

$^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$, $^{\S\S\S}p<0.001$ significantly different compared to OI 25 mg/kg.

$^{ff}p<0.01$, $^{fff}p<0.001$ significantly different compared to OI 50 mg/kg.

$^{\#}p<0.05$ significantly different compared to IND 10 mg/kg.

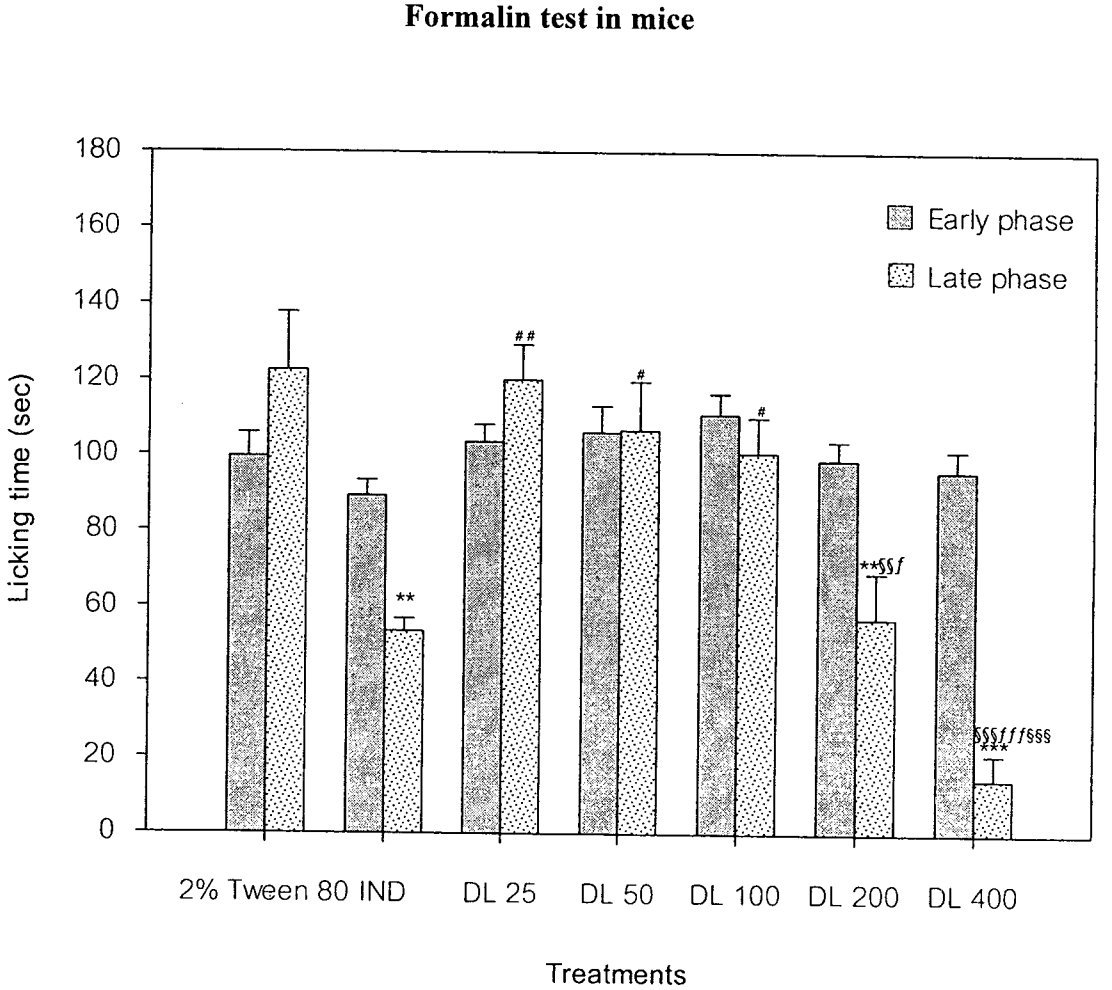


Figure 82 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg). N=8 for all groups.

$^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{ƒ}p<0.05$, $^{ƒƒƒ}p<0.001$ significantly different compared to DL 50 mg/kg.

$^{§§}p<0.01$, $^{§§§}p<0.001$ significantly different compared to DL 25 mg/kg.

$^{§§§}p<0.001$ significantly different compared to DL 100 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$ significantly different compared to IND 10 mg/kg.

Formalin test in mice

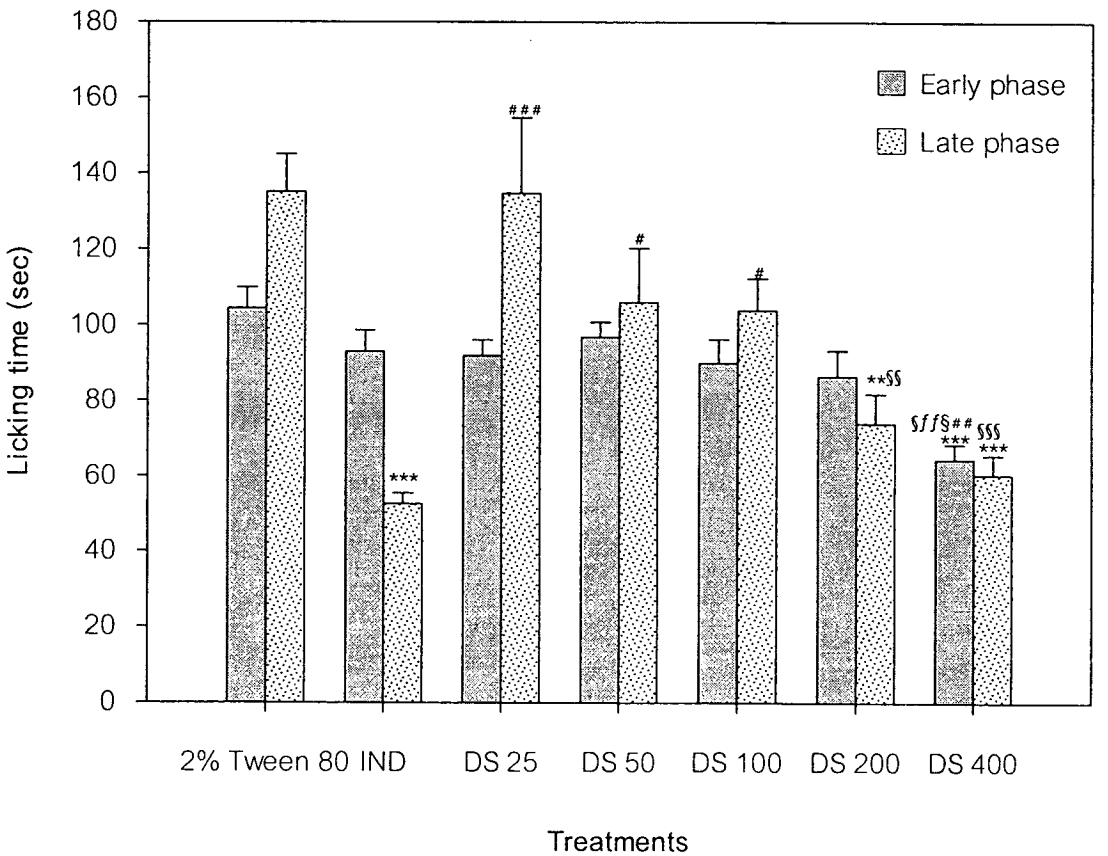


Figure 83 Time spent on hind paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). N=8 for all groups.

$^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$ significantly different compared to DS 100 mg/kg.

$^{\S}p<0.05$, $^{\S\S}p<0.01$, $^{\S\S\S}p<0.001$ significantly different compared to DS 25 mg/kg.

$^{ff}p<0.01$ significantly different compared to DS 50 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

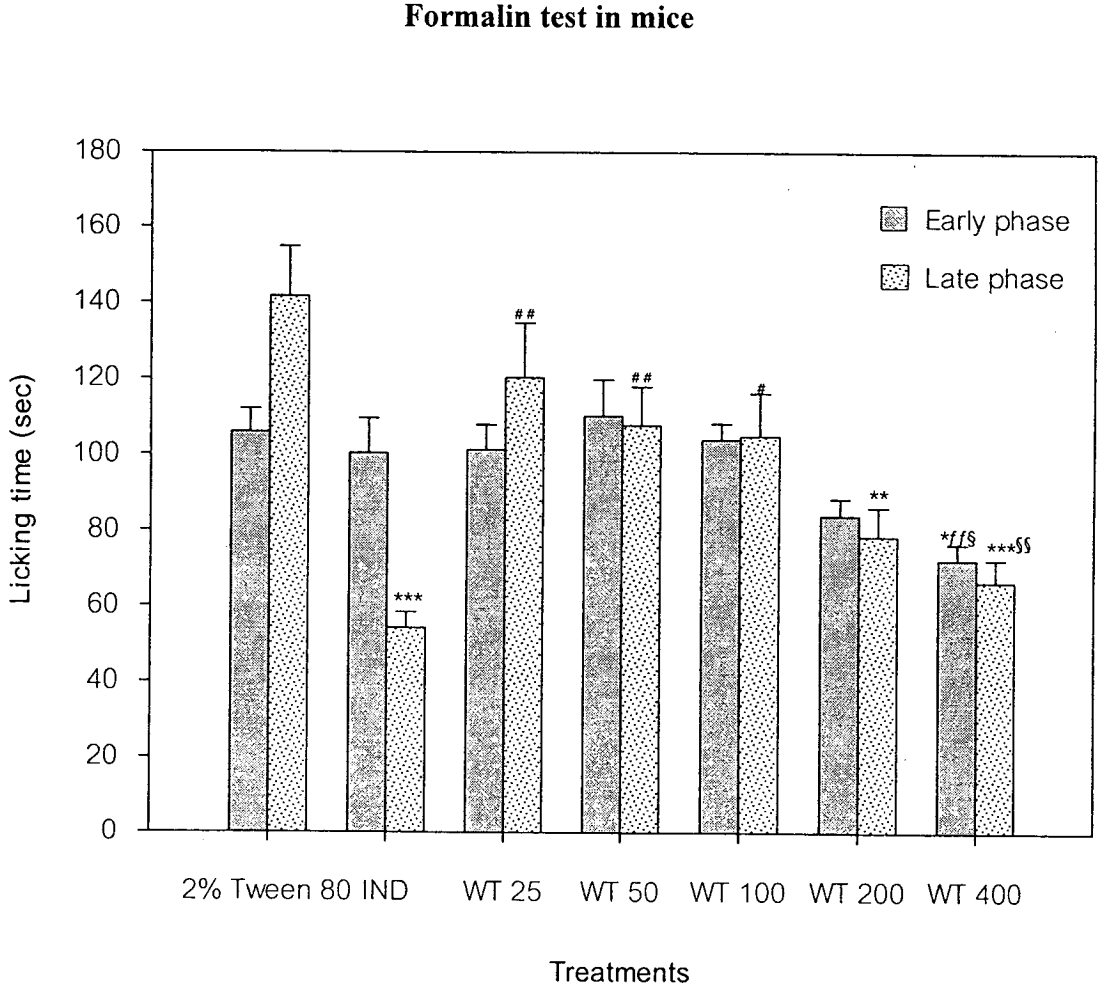


Figure 84 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Walsura trichostemon* root extract (WT; 25-400 mg/kg). N=8 for all groups.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$ significantly different compared to WT 100 mg/kg.

$^{\S\S}p<0.01$ significantly different compared to WT 25 mg/kg.

$^{ff}p<0.01$ significantly different compared to WT 50 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$ significantly different compared to IND 10 mg/kg.

Table 43 Time spent on paw licking after intraperitoneal administration of 0.9% normal saline solution (NSS; 10 ml/kg) and morphine sulfate (MO; 10 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatment	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
NSS		81.92±8.60	158.80±6.08
MO	10	7.48±1.84*** (90.87%)	3.67±1.08*** (97.69%)

***$p<0.001$ significantly different compared to NSS.

Table 44 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		113.56±4.83	158.66±13.07
IND	10	100.61±4.33 (-11.40%)	68.89±10.85** (-56.58%)
BMY	125	96.67±4.97 (-14.87%)	80.97±18.20** (-48.96%)
	250	90.82±6.93* (-20.03%)	68.47±14.99** (-56.85%)
	500	93.20±3.89 (-17.93%)	77.27±18.43** (-51.30%)

*$p<0.05$, **$p<0.01$ significantly different compared to 2% Tween 80.

Table 45 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Aegle marmelos* root extract (AM; 25-400 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		98.06±5.45	145.57±17.16
IND	10	95.98±10.65 (-2.12%)	55.73±5.83*** (-61.71%)
AM	25	103.42±6.64 (5.45%)	161.76±8.25### (11.12%)
	50	94.09±10.62 (-4.05%)	133.80±9.44### (-8.08%)
	100	98.60±4.03 (0.55%)	132.43±9.26### (-9.02%)
	200	85.68±7.03 (-12.62%)	118.91±9.70## (-18.31%)
	400	64.19±4.08*§§ (-34.55%)	58.44±5.50***§§§¶ (-59.85%)

*$p<0.05$, **$p<0.01$ significantly different compared to 2% Tween 80.

§$p<0.05$ significantly different compared to AM 100 mg/kg.

§§$p<0.01$ significantly different compared to AM 25 mg/kg.

¶¶$p<0.01$ significantly different compared to AM 200 mg/kg.

##$p<0.01$, ###$p<0.001$ significantly different compared to IND 10 mg/kg.

§§§$p<0.001$ significantly different compared to AM 25-100 mg/kg.

Table 46 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		110.03±10.08	144.41±10.68
IND	10	85.31±6.77 (-22.47%)	73.70±2.00** (-48.96%)
OI	25	109.31±5.63 (-0.65%)	128.02±12.38# (-11.35%)
	50	105.48±7.62 (-4.13%)	118.32±6.67 (-18.06%)
	100	107.74±6.40 (-2.08%)	73.07± 9.98**§ (-49.40%)
	200	107.65±4.31 (-2.16%)	55.18±9.03***§§§ ††† (-61.78%)
	400	112.52±12.87 (2.26%)	45.80±17.08***§§§ ††† (-68.28%)

**$p<0.01$, ***$p<0.001$ significantly different compared to 2% Tween 80.

§$p<0.05$, §§§$p<0.001$ significantly different compared to OI 25 mg/kg.

††$p<0.01$, †††$p<0.001$ significantly different compared to OI 50 mg/kg.

#$p<0.05$ significantly different compared to IND 10 mg/kg.

Table 47 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg). Each value represents mean±S.E.M., N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		99.55±6.25	122.54±15.26
IND	10	89.21±4.14 (-10.39%)	53.44±3.40** (-56.39%)
DL	25	103.56±4.56 (4.02%)	120.08±9.32## (-2.01%)
	50	106.04±7.11 (6.52%)	106.66±13.05# (-12.96%)
	100	110.94±5.62 (11.44%)	100.72±9.42# (-17.81%)
	200	98.77±5.04 (-0.78%)	56.90±12.12**§§ʃ (-53.57%)
	400	95.92±5.47 (-3.64%)	14.52±6.54***§§§ʃʃʃ§§§ (-88.14%)

**$p<0.01$, ***$p<0.001$ significantly different compared to 2% Tween 80.

§§$p<0.01$, §§§$p<0.001$ significantly different compared to DL 25 mg/kg.

ʃ$p<0.05$, ʃʃʃ$p<0.001$ significantly different compared to DL 50 mg/kg.

§§§$p<0.001$ significantly different compared to DL 100 mg/kg.

#$p<0.05$, ##$p<0.01$ significantly different compared to IND 10 mg/kg.

Table 48 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		104.38±5.50	135.07±10.01
IND	10	93.00±5.66 (-10.89%)	52.48±2.98*** (-61.15%)
DS	25	91.88±4.18 (-11.97%)	134.68±19.98### (-0.29%)
	50	96.84±3.94 (-7.22%)	105.98±14.25# (-21.53%)
	100	89.92±6.32 (-13.84%)	103.88±8.44# (-23.10%)
	200	86.42±6.84 (-17.20%)	73.99±7.81***§§§ (-45.22%)
	400	64.53±3.99***§/§*** (-38.17%)	60.36±5.32**§§§ (-55.31%)

**$p<0.01$, ***$p<0.001$ significantly different compared to 2% Tween 80.

§$p<0.05$ significantly different compared to DL 100 mg/kg.

§$p<0.05$, §§$p<0.01$, §§§$p<0.001$ significantly different compared to DL 25 mg/kg.

//$p<0.01$ significantly different compared to DL 50 mg/kg.

#$p<0.05$, ##$p<0.01$, ###$p<0.001$ significantly different compared to IND 10 mg/kg.

Table 49 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Walsura trichostemon* root extract (WT; 25-400 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		105.03±6.11	141.59±13.19
IND	10	100.38±9.15 (-5.13%)	54.16±4.17*** (-61.74%)
WT	25	101.28±6.64 (-4.28%)	120.20±14.52$^{\#\#}$ (-15.10%)
	50	110.20±9.60 (4.14%)	107.85±10.11$^{\#\#}$ (-23.82%)
	100	104.08±4.19 (-1.64%)	104.98±11.27$^{\#}$ (-25.85%)
	200	84.05±4.46 (-20.57%)	78.59±7.71** (-44.49%)
	400	72.32±4.15$^{*\,\S\S}$ (-31.66%)	66.55±5.82***§§ (-53.00%)

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$ significantly different compared to DL 100 mg/kg.

$^{\S\S}p<0.01$ significantly different compared to DL 25 mg/kg.

$^{\S\S}p<0.01$ significantly different compared to DL 50 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$ significantly different compared to IND 10 mg/kg.

Acetic-acid induced writhes in mice

The acetic acid-induced writhing method to examine the analgesic efficacy of BMY and five herbal root extracts (AM, OI, DL, DS and WT). Each mouse was administered orally 2% Tween 80, indomethacin (IND; 10 mg/kg), various doses of BMY (125, 250, 500 mg/kg) or AM, OI, DL, DS and WT (25, 50, 100, 200, 400 mg/kg).

To demonstrate the validity of acetic acid-induced writhing method, IND 10 mg/kg was used as a positive control. As expected IND significantly ($p<0.001$) decreased writhing response by 85.48% compared with 2%Tween 80. All doses of BMY (125, 250 and 500 mg/kg) significantly ($p<0.05$, $p<0.01$, $p<0.001$, respectively) decreased the number of writhes induced by acetic acid by 32.78%, 43.57% and 59.34%, respectively when compared to vehicle control. The antinociceptive efficacy of BMY 500 mg/kg is comparable to IND (Figure 85).

AM at doses of 200, 400 mg/kg significantly ($p<0.001$) decreased the number of writhes induced by acetic acid by 69.36% and 87.54%, respectively when compared to vehicle control. AM at doses of 200, 400 mg/kg significantly ($p<0.001$) decreased the number of writhes when compared to AM (25, 50 and 100 mg/kg). IND significantly ($p<0.001$) decreased writhing response by 83.16% compared with 2% Tween 80. The antinociceptive efficacy of AM 200 and 400 mg/kg are comparable to IND (Figure 86).

OI at doses of 100, 200, 400 mg/kg significantly ($p<0.05$, $p<0.05$ and $p<0.001$, respectively) decreased the number of writhes induced by acetic acid by 33.84%, 37.16% and 58.31%, respectively when compared to vehicle control. IND significantly ($p<0.001$) decreased the number of writhes by 78.55% when compared to vehicle control. The antinociceptive efficacy of OI 400 mg/kg is comparable to IND (Figure 87).

DL at doses of 100, 200, 400 mg/kg significantly ($p<0.05$, $p<0.01$, $p<0.001$, respectively) decreased the number of writhes by 33.55%, 38.98% and 67.73%, respectively when compared to vehicle control. DL 400 mg/kg significantly ($p<0.01$,

$p<0.01$ and $p<0.05$, respectively) decreased the number of writhes when compared to DL at doses of 25, 50 and 100 mg/kg. IND significantly decreased the number of writhes by 75.40% when compared to vehicle control. The antinociceptive efficacy of DL 400 mg/kg is comparable to IND (Figure 88).

DS at doses of 200, 400 mg/kg significantly ($p<0.05$ and $p<0.01$, respectively) decreased the number of writhes by 37.18% and 45.49%, respectively when compared to vehicle control. DS 400 significantly ($p<0.05$) decreased the number of writhes when compared to DS 25 mg/kg. IND significantly ($p<0.001$) decreased the number of writhes by 85.92% when compared to vehicle control. IND has higher antinociceptive efficacy than DS 25-400 mg/kg (Figure 89).

WT at doses of 200, 400 mg/kg significantly ($p<0.001$) decreased the number of writhes by 49.98 % and 61.50%, respectively when compared to vehicle control. WT 200 mg/kg significantly decreased the number of writhes ($p<0.001$, $p<0.01$, respectively) when compared to WT 25 and 50 mg/kg. WT 400 mg/kg significantly ($p<0.001$ and $p<0.01$, respectively) decreased the number of writhes when compared to WT 50 and 100 mg/kg. IND significantly ($p<0.001$) decreased the number of writhes by 93.52% when compared to vehicle control. IND has higher antinociceptive efficacy than WT 25-400 mg/kg (Figure 90).

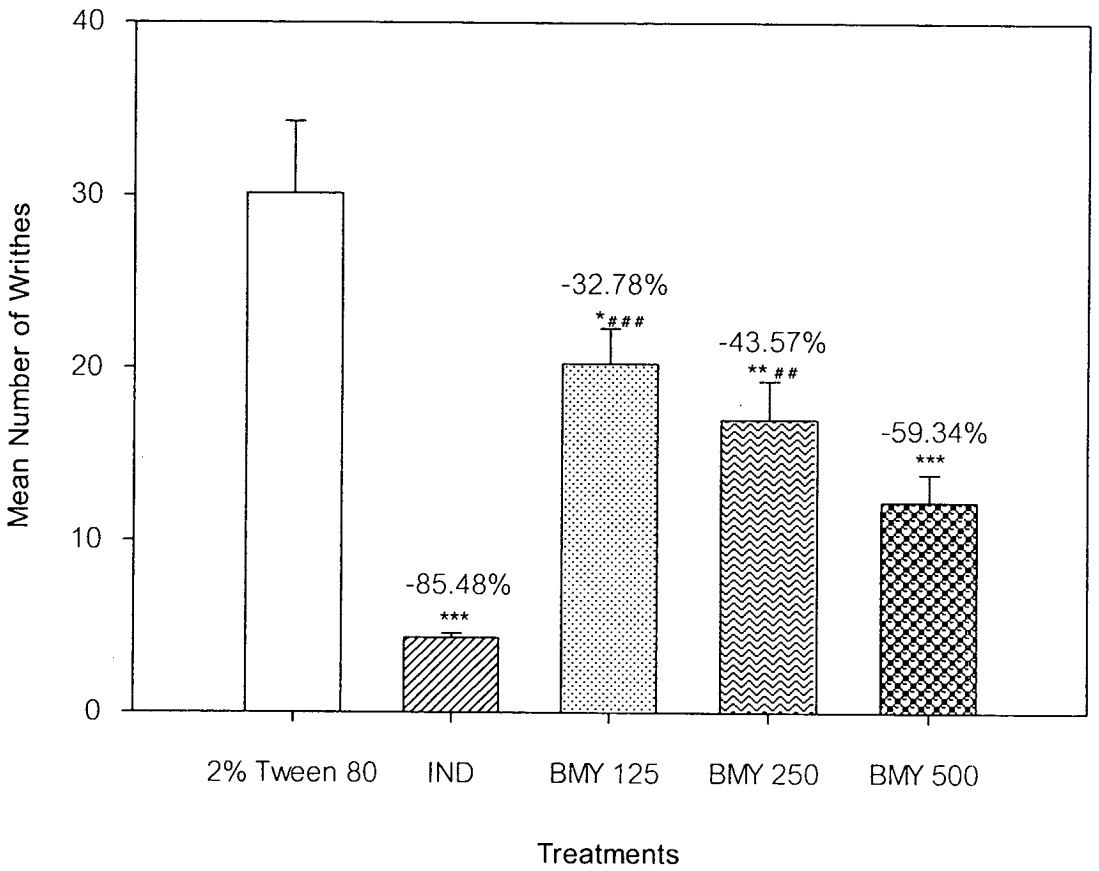


Figure 85 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=8 for all groups.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.
$^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Acetic acid-induced writhing in mice

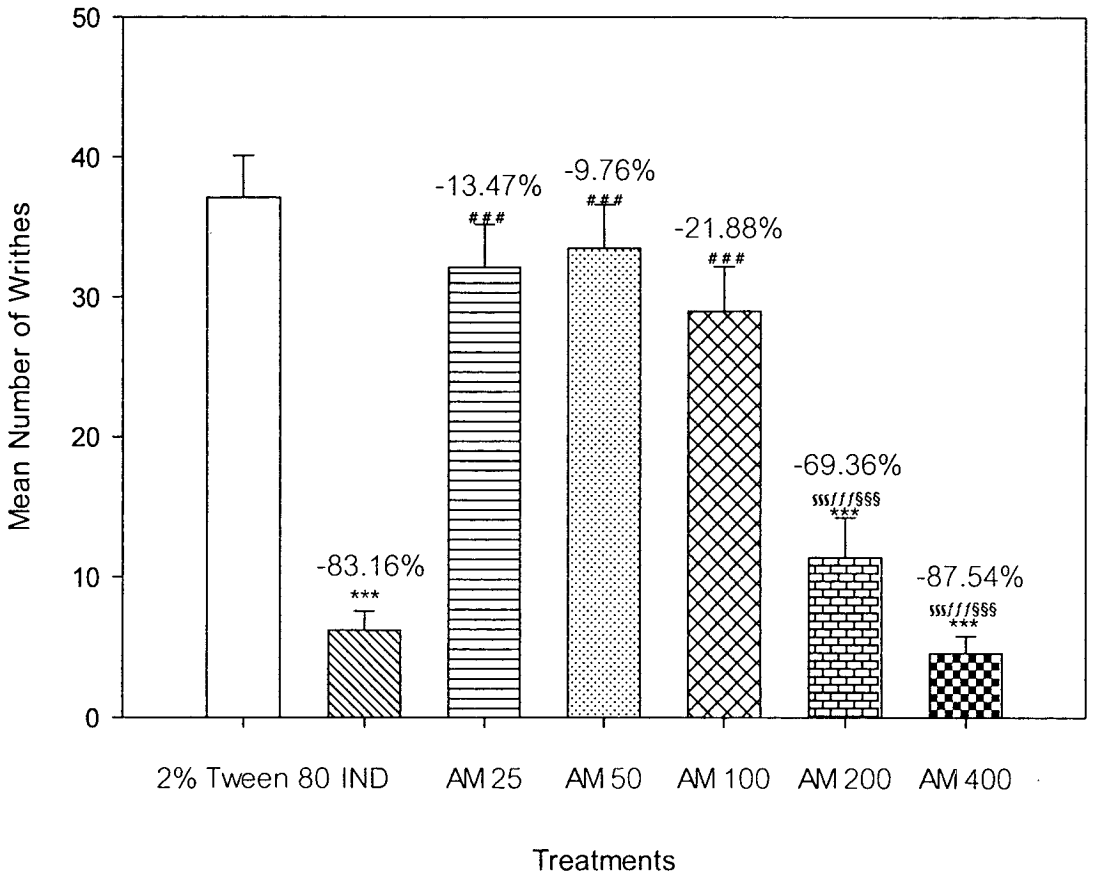


Figure 86 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Aegle marmelos* root extract (AM; 25-400 mg/kg). N=8 for all groups.

$^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{§§§}p<0.001$ significantly different compared to AM 25 mg/kg.

$^{ʃʃʃ}p<0.001$ significantly different compared to AM 50 mg/kg.

$^{§§§}p<0.001$ significantly different compared to AM 100 mg/kg.

$^{###}p<0.001$ significantly different compared to IND 10 mg/kg.

Acetic acid-induced writhing in mice

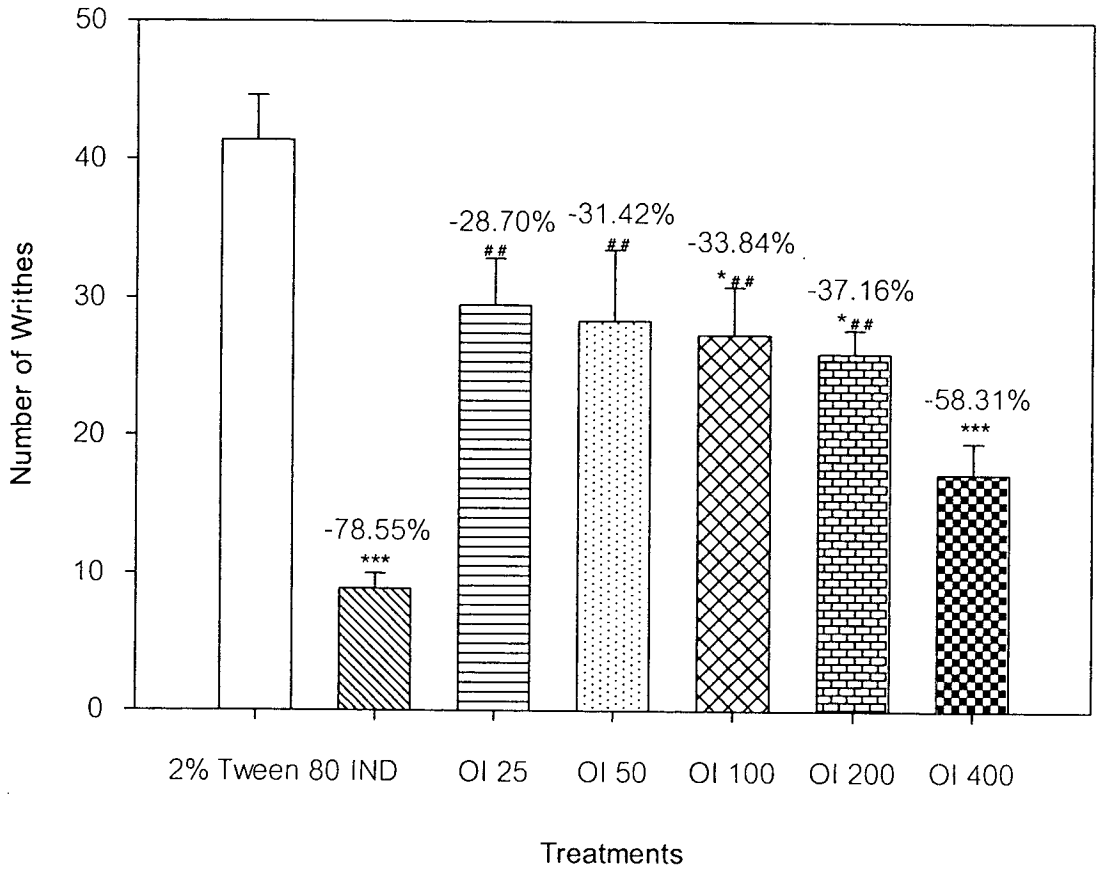


Figure 87 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg). N=8 for all groups.

$^{*}p<0.05$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\#\#}p<0.01$ significantly different compared to IND 10 mg/kg.

Acetic acid-induced writhing in mice

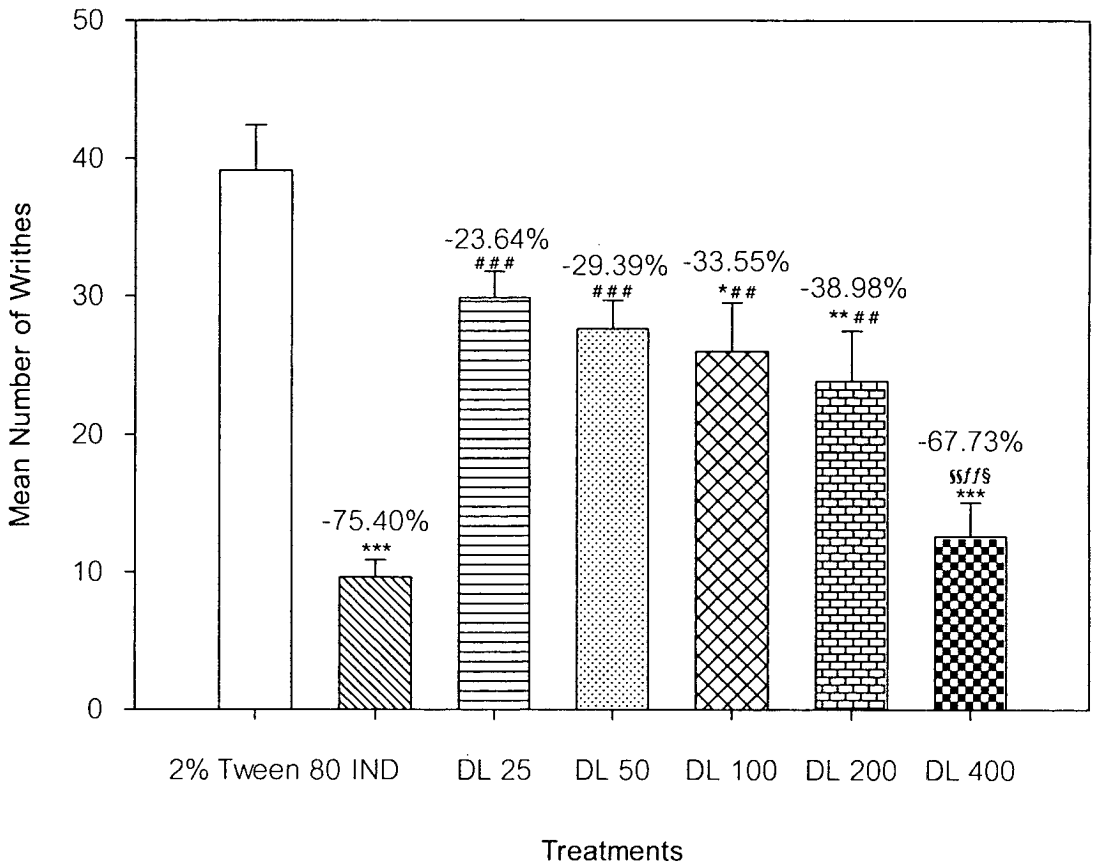


Figure 88 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg). N=8 for all groups.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$ significantly different compared to DL 100 mg/kg.

$^{\S\S}p<0.01$ significantly different compared to DL 25 mg/kg.

$^{ff}p<0.01$ significantly different compared to DL 50 mg/kg.

$^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Acetic acid-induced writhing in mice

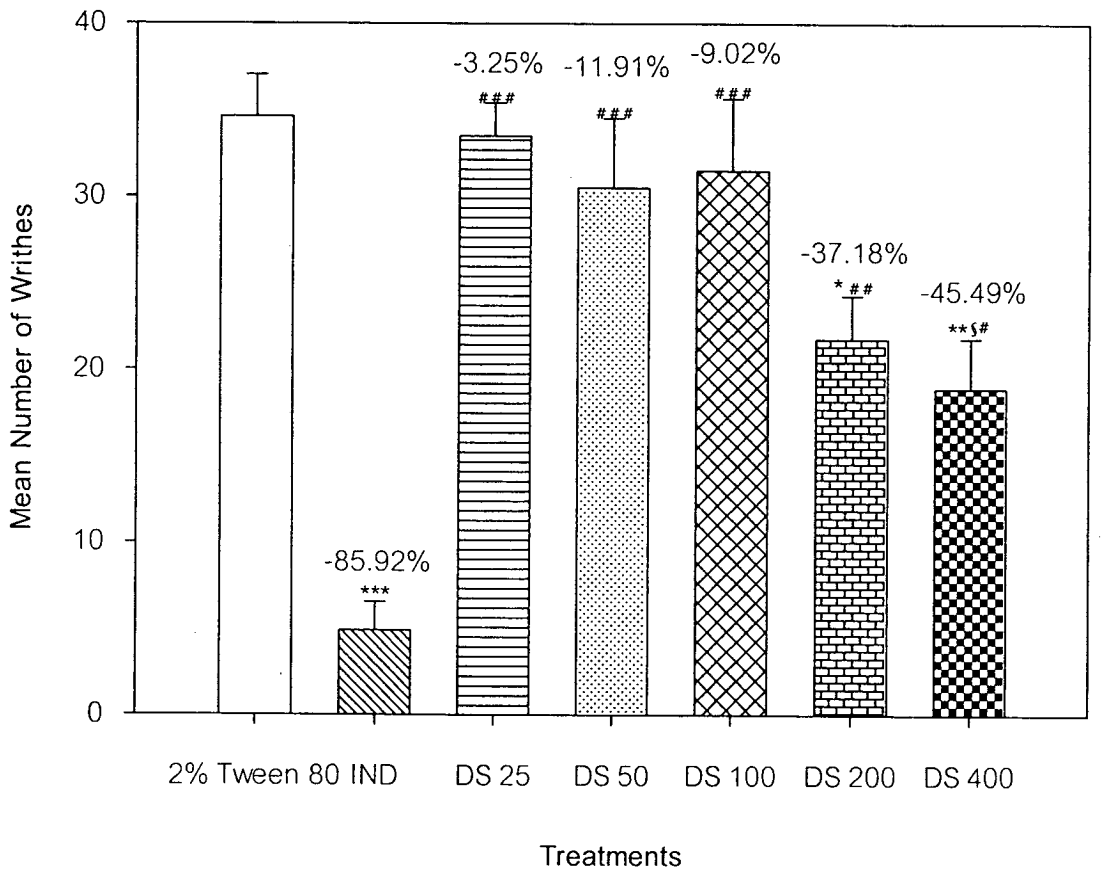


Figure 89 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). N=8 for all groups.

$^{*}p<0.05$, $^{**}p<0.01$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$ significantly different compared to DS 25 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Acetic acid-induced writhing in mice

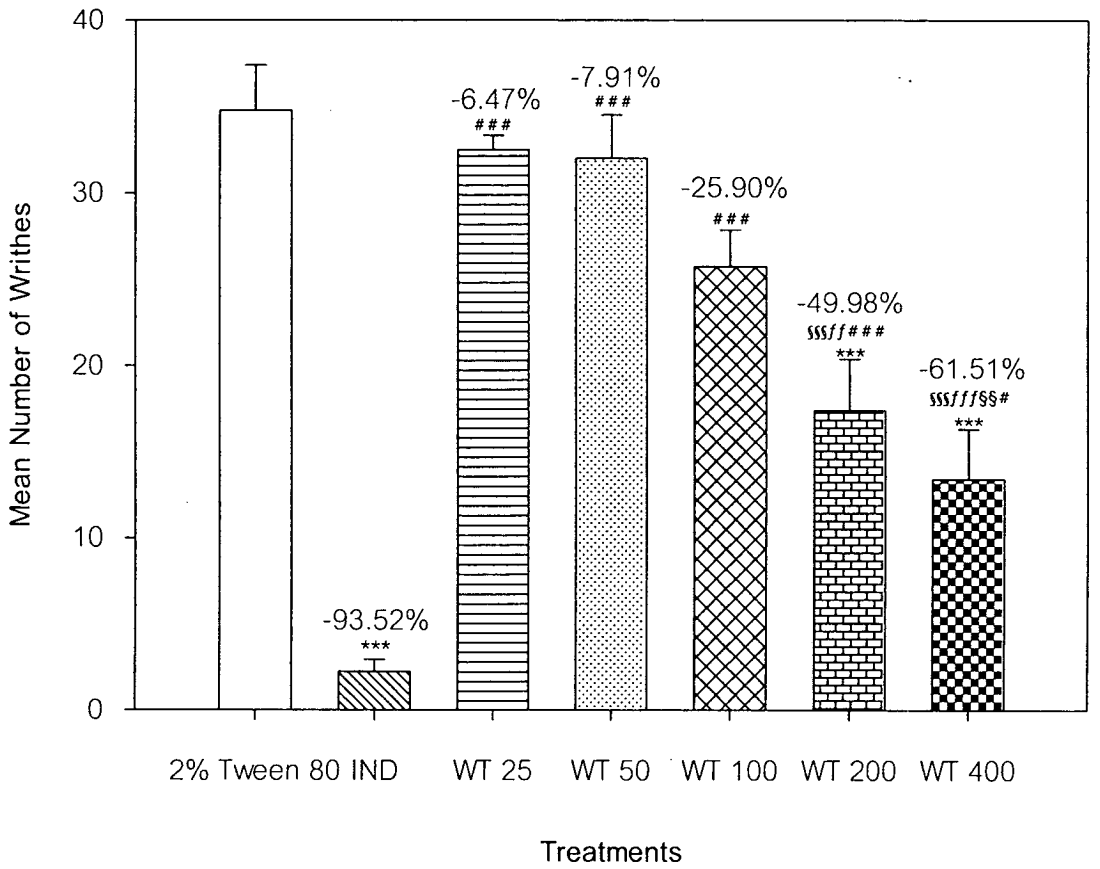


Figure 90 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Walsura trichostemon* root extract (WT; 25-400 mg/kg). N=8 for all groups.

$^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S\S}p<0.01$ significantly different compared to WT 100 mg/kg.

$^{fff}p<0.01$, $^{fff}p<0.001$ significantly different compared to WT 50 mg/kg.

$^{\S\S\S}p<0.001$ significantly different compared to WT 25 mg/kg.

$^{\#}p<0.05$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Rota-rod performance test in mice

In order to determine the effect of BMY and five herbal root extracts (AM, OI, DL, DS and WT) on motor response, mice were administered 2% Tween 80, BMY (500 mg/kg), AM, OI, DL, DS or WT (400 mg/kg) orally and tested on the rota-rod apparatus for 5 subsequent trials at 30, 60, 90, 120 and 240 min after drug administration. The results showed that BMY, AM, OI, DL, DS and WT at doses tested did not affect the motor response of the animals (Figure 91).

Rota-rod performance test in mice

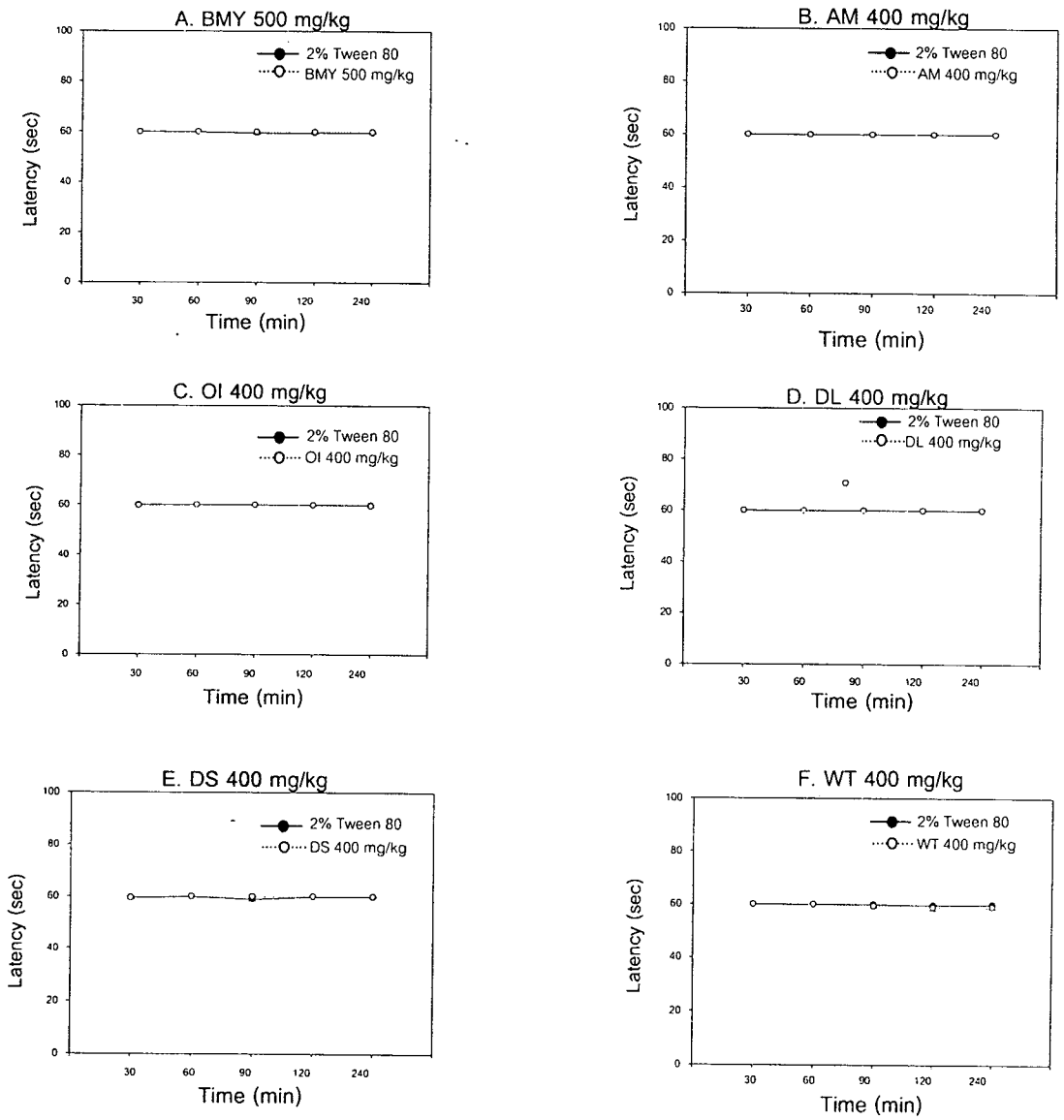


Figure 91 Rota-rod latency of each extract after oral administration compared to 2% Tween 80 (10 ml/kg). N=8 for all groups.

A. Ben-Cha-Moon-Yai remedy extract (BMY; 500 mg/kg). B. *Aegle marmelos* root extract (AM; 400 mg/kg). C.*Oroxylum indicum* root extract (OI; 400 mg/kg). D. *Dimocarpus longan* root extract (DL; 400 mg/kg). E. *Dolichandrone serrulata* root extract (DS; 400 mg/kg). F. *Walsura trichostemon* root extract (WT; 400 mg/kg).