PHYTOCHEMICAL STUDY, ANTIMICROBIAL AND ANTIANGIOGENIC ACTIVITIES OF THE LEAF EXTRACTS OF *GYNURA SEGETUM*

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by

SEOW LAY JING

Thesis submitted in fulfillment of the requirements for the Degree of Master of Science

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CAM	Chick embryo chorioallantoic membrane
cm	Centimeter
DMSO	Dimethyl sulfoxide
eV	Electron volt
g	Gram
GC-MS	Gas chromatography-mass spectrometry
HCl	Hydrochloric acid
HL-60	Human promyelocytic leukemia cells
HPTLC	High performance thin layer chromatography
i.d.	Internal diameter
kg	Kilogram
m	Meter
mg	Milligram
mg/ml	Milligrams per milliliter
ml	Milliliter
ml/min	Milliliters per minute
mm	Millimeter
MIC	Minimum inhibitory concentration
NaOH	Sodium hydroxide
nm	Nanometer

NP/PEG	Natural products reagent
ppm	Part per million
R_f	Retardation factor
S.D.	Standard deviation
TLC	Thin layer chromatography
UV	Ultraviolet
v/v	Volume to volume
µg	Microgram
µl	Microliter
µm	Micrometer
°C	Degree Celsius

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KAJIAN FOTOKIMIA, AKTIVITI ANTIMIKROBIAL DAN ANTIANGIOGENIK UNTUK EKSTRAK DAUN *GYNURA SEGETUM*

ABSTRAK

Gynura segetum (daun dewa dalam bahasa Malaysia) ialah herba daripada keluarga compositae. Tumbuhan ini dipercayai berguna untuk mengubati kanser, kencing manis, darah tinggi, radang dan jangkitan kulit. Daun *Gynura segetum* dinilai menggunakan parameter kawalan mutu yang termasuk analisis gravimetri, kromatografi lapisan nipis (TLC) dan kromatografi cecair prestasi tinggi (HPLC).

Aktiviti antimikrobial ekstrak pelbagai pelarut daun *Gynura segetum* telah dinilaikan dengan kaedah resapan agar. Fraksi etil asetat menunjukkan kesan perencatan yang paling tinggi dan oleh itu pemfraksian selanjutnya telah dijalankan ke atas fraksi ini. Lima belas subfraksi baru yang diperolehi telah diuji untul aktiviti antimikrobial dan kepekatan perencatan minimum (MIC) untuk subfraksi yang aktif diselidiki dengan menggunakan kaedah pencairan tabung. Di antara subfraksi yang diuji, E4 menunjukkan aktiviti antimikrobial yang tertinggi. Analisis kromatografi gas-spektrometer jisim (GC-MS) telah dilakukan untuk mengenal pasti kandungan kimia sampel yang aktif. Lima belas sebatian yang dikenali yang terdapat dalam fraksi etil asetat dan subfraksi E4 telah dikenalpasti mengunakan analisis GCMS didapati ialah

metil ester, asid butanadioik (63), 4-vinil fenol (64), niasin (65), 1-tetradekena (66), fenol, 2,4-bis(1,1-dimetil etil) (67), 1-heksadekena (68), 4-hidroksi asid benzoik (69), *E*-15-heptadekenal (70), asid heksadekanoik (53), 1,2-dibutil ester, asid karboksilik benzena (71), 1-dokosena (72), asid oktadekanoik (73), 1-eikosena (74), siklotetrakosana (75) dan bis (2etil heksil) ester, 1,2 asid karboksilik benzena (76). Keputusan daripada kajian in mencadangkan hubungan diantara aktiviti antimikrobial dengan struktur kimia. Kehadiran rantai alifatik dan kumpulan hidroksi (OH) bertanggungjawab untuk aktiviti antimikrobial daun *Gynura segetum*.

Aktiviti antiangiogenik ekstrak dan fraksi daripada daun *Gynura segetum* telah dinilaikan *in vivo* dengan menggunakan kaedah membran korioalantoik (CAM) embrio ayam. Ekstrak dan fraksi daun *Gynura segetum* menunjukkan kesan antiangiogenik yang lebih baik daripada suramin. Analisis kimia untuk sampel yang aktif daripada daun *Gynura segetum* menghasilkan sembilan sebatian yang dikenali, iaitu undekana (77), neophytadine (78), metil ester, asid heksadekanoik (79), metil ester, 9,12 asid oktadekadienoik (80), metil ester, 9,12,15 asid oktadekatrienoik (81), fitol (21), tetradekanal (82), metil ester, asid oktadekadienoik (83) and γ-sitosterol (84). Penemuan ini dengan jelasnya mencadangkan bahawa kehadiran asid lemak dan sebatian sterol dalam sampel yang aktif daun *Gynura segetum* mungkin bertanggungjawab untuk aktiviti antiangiogenik dan berpotensi sebagai sumber tambahan untuk rawatan kanser.

ABSTRACT

Gynura segetum (daun dewa in Malay) is a herb from family compositae, reputed to possess various medicinal values. This plant is believed of value for treating cancer, diabetes, hypertension, inflammation and skin infection. The leaves of *Gynura segetum* were evaluated using quality control parameters, which include gravimetric, thin layer chromatographic (TLC) and high-performance liquid chromatographic (HPLC) analysis.

The antimicrobial activity of different solvent extracts of leaves of *Gynura segetum* was evaluated using agar diffusion method. Ethyl acetate fraction exhibited the highest inhibitory effect and therefore further fractionation on this fraction was carried out. Fifteen new subfractions obtained were tested for antimicrobial activity and the MIC of the active subfractions was determined by tube dilution method. Among the subfractions tested, E4 showed the highest antimicrobial activity. Gas Chromatography-mass spectrometry (GC-MS) analysis was carried out to identify the chemical compositions of the active sample. Fifteen known compounds in ethyl acetate fraction and subfraction E4 were identified by GCMS analysis butanedioic acid, monomethyl ester (63), 4-vinylphenol (64), niacin (65), 1-tetradecene (66), phenol, 2,4-bis(1,1-dimethylethyl) (67), 1-hexadecene (68), 4-hydroxy-benzoic acid (69), E-15-heptadecenal (70), hexadecanoic acid (53), 1,2-benzenedicarboxylic acid, dibutyl ester (71), 1-docosene (72), octadecanoic acid (73), 1-eicosene (74), cyclotetracosane (75),

1,2-benzenedicarboxylic acid, bis(2-ethylhexyl)ester (76). The result of this study suggested that the related of the antimicrobial activity with chemical structures. The presence of the aliphatic chains and the hydroxyl (OH) group were responsible for the antimicrobial activity of *G. segetum* leaves.

The antiangiogenic activity of *Gynura segetum* leaves extracts and its fractions was evaluated *in vivo* using the chick embryo chorioallantoic membrane (CAM) assay. The *Gynura segetum* leaves extracts and its fractions showed a significantly greater antiangiogenic effect compared to suramin. Chemical analysis of the active extracts from the leaves of *Gynura segetum* yielded nine known compounds: undecane (77), neophytadine (78), hexadecanoic acid, methyl ester (79), 9,12-octadecadienoic acid, methyl ester (80), 9,12,15-octadecatrienoic acid, methyl ester (81), phytol (21), tetradecanal (82), octadecanoic acid, methyl ester (83) and γ-sitosterol (84). These findings suggest the occurrence of the fatty acids and sterol compounds in the active sample of leaves of *Gynura segetum* might be responsible in antiangiogenic activity and may be a potential supplemental source for cancer treatment.

CHAPTER 1

INTRODUCTION

1.1 General

Herbs were the earliest source of medicine. The use of medicinal plants for healing dates as far back as prehistoric times and has since been woven into the culture and civilization of people (Indu & Ng, 2000). Plants contain phytochemical that can be used for therapeutic purpose or as precursors for pharmaceutical synthesis (Makut *et al.*, 2008).

According to World Health Organization (WHO), approximately 80% of the world population currently depends on traditional treatments and this number is in the increase even in young people. In accordance with this, the world market demand for herbs and their products has increased tremendously in recent years (Indu & Ng, 2000). The global awakening of herbal medicine is also reflected in Malaysia, where the demand for medicinal products has increased over the past years.

Malaysia being a tropical rainforest is considered one of the most evolved and diverse rainforests in the world which supports more than 20,000 plant species. The number of medicinal plants used in Malaysia is estimated more than the 2000 species, indicating that the supportive environment of Malaysia for the sustainable development of medicinal plant.

The compositae (asteracea) is the largest family of flowering plants and contains about 900 genera and 13000 species. The family consists of aromatic annual or short lived glandular and hairy herbs and shrubs but rarely trees. Leaves are usually alternate, inflorescence is a centripetal head of usually many small flowers. Fruits are generally grow directly from the stem or peduncle of a plant. Chemical and medicinal research in recent years has increased interest in this family and we now have a better knowledge of many almost-discarded folk medicines as well as hitherto uninvestigated plants (Trease & Evans, 1978).

In Malaysia, the use of *Gynura segetum* is only confined to the traditional healers and the midwives. There is no commercial product of this plant in the local market. However, in countries such as China and Indonesia, the potentials of this plant was long recognized and tapped.

1.2 Phytochemical and biological activities of genus *Gynura*

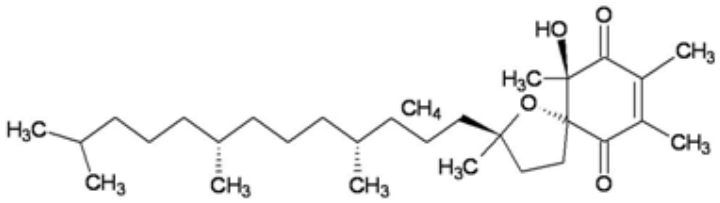
The *Gynura* species are occurring in East Asia and the Himalayas (Davies, 1979). Different *Gynura* species have proved to be of special significance among phytochemists in recent years because they have been found to possess a number of biological activities. The chemical constituents of different *Gynura* species have been studied for over a 20 years. Pyrrolizidine alkaloids and flavonoids are commonly found to occur in this genus.

A review of the literature indicates that several *Gynura* species products and their extract have been used for the treatment of diseases. It is evident that phytochemicals or the active constituents from genus *Gynura* have a potential to act as useful medicine.

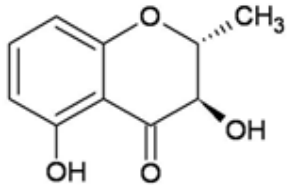
1.2.1 *Gynura japonica*

Lin and co-workers (2003) had elucidated a novel quinonoid terpenoid: (-)-α-tocospirone (1), a new chromanone: (-)-gynuraone (2), as well as three new steroids, (22*E*,24*S*)-7α-hydroperoxystigmasta-5,22-dien-3β-ol (3), (22*E*,24*S*)-stigmasta-1,4,22-trien-3-one (4), and (24*R*)-stigmasta-1,4-dien-3-one (5), together with 15 known components, from the rhizome of Formosan *G. japonica*. Among the isolates, caryophyllene oxide (6), 6-acetyl-2, 2-dimethylchroman-4-one (7), vanillin (8), 2, 6-dimethoxy-1, 4-benzoquinone (9), and benzoic acid (10) exhibited significant anti-platelet aggregation activity *in vitro*. The structures of compounds are shown in Figure 1.1.

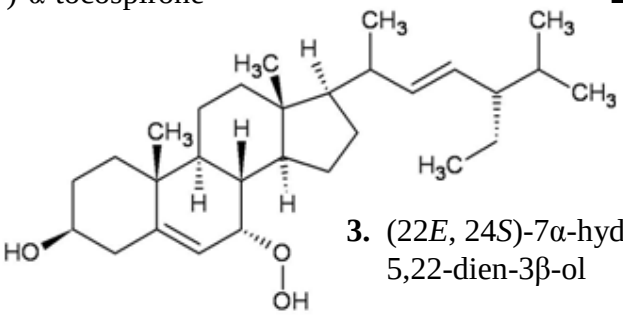
Four new cerebrosides, gynuramides, together with 37 known compounds, were isolated from the rhizome of *G. japonica* (Lin *et al.*, 2004). The structures of cerebrosides were detected by spectroscopic examination as to be (2*S*,3*S*,4*R*,8*E*)-2-[(*R*)-2-hydroxypentacosanoylamino]-8-en-1,3,4-octadecanetriol (11), (2*S*,3*S*,4*R*,8*E*)-2-[(*R*)-2-hydroxytetracosanoylamino]-8-en-1,3,4-octadecanetriol (12), (2*S*,3*S*,4*R*,8*E*)-2-[(*R*)-2-hydroxytricosanoylamino]-8-en-1,3,4-octadecanetriol (13), and (2*S*,3*S*,4*R*,8*E*)-2-[(*R*)-2-hydroxydocosanoylamino]-8-en-1,3,4-octadecanetriol (14), respectively and their structures shown in Figure 1.2.



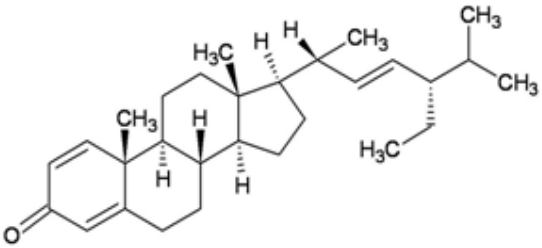
1. (-)-α-tocospirone



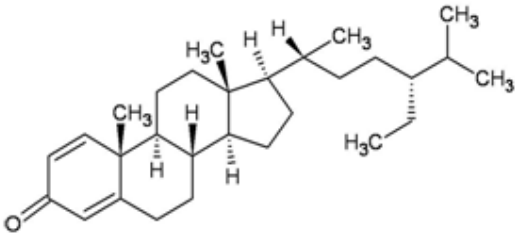
2. (-)-gynuraone



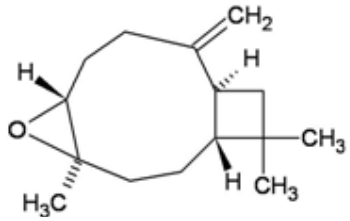
3. (22*E*, 24*S*)-7α-hydroperoxystigmasta-5,22-dien-3β-ol



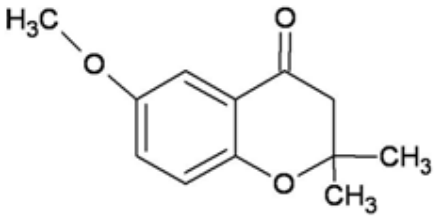
4. (22*E*, 24*S*)-stigmasta-1,4,22-trien-3-one



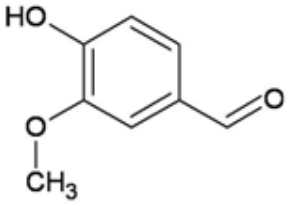
5. (24*R*)-stigmasta-1,4-dien-3-one



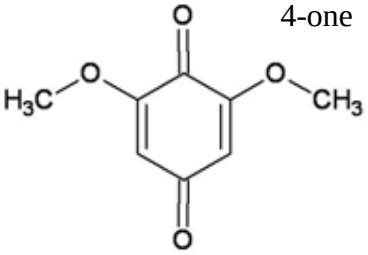
6. caryophyllene oxide



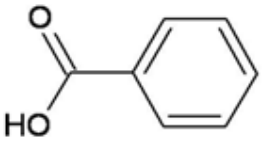
7. 6-acetyl-2, 2-dimethylchroman-4-one



8. vanillin

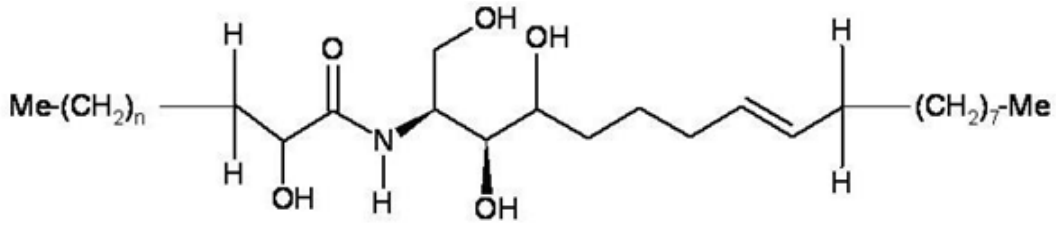


9. 2, 6-dimethoxy-1, 4-benzoquinon



10. benzoic acid

Figure 1.1 Compounds from the rhizome of *Gynura japonica*



11. n=21 (2*S*,3*S*,4*R*,8*E*)-2-[(*R*)-2-hydroxypentacosanoylamino]-8-en-1,3,4-octadecanetriol

12. n=20 (2*S*,3*S*,4*R*,8*E*)-2-[(*R*)-2-hydroxytetracosanoylamino]-8-en-1,3,4-octadecanetriol

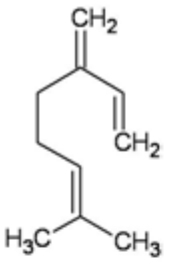
13. n=19 (2*S*,3*S*,4*R*,8*E*)-2-[(*R*)-2-hydroxytricosanoylamino]-8-en-1,3,4-octadecanetriol

14. n=18 (2*S*,3*S*,4*R*,8*E*)-2-[(*R*)-2-hydroxydocosanoylamino]-8-en-1,3,4-octadecanetriol

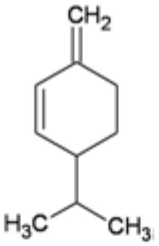
Figure 1.2 The structures of new cerebrosides from the rhizome of *G. japonica*

1.2.2 *Gynura cusimbua*

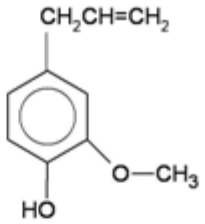
Forty-seven constituents totaling 91.92% of the oil were isolated from the aerial parts of *G. cusimbua* by Rana and Blazquez (2007). The major constituents of the oil (Figure 1.3) are myrcene (31.0%) (15), β-phellandrene (12.43%) (16), eugenol (6.34%) (17), α-humulene (6.20%) (18), dodecyl acrylate (6.09%) (19), α-copaene (5.61%) (20), phytol (3.21%) (21), germacrene D (3.0%) (22), cryptone (2.04%) (23), 2,4-ditertbutylphenol (1.62%) (24), α-pinene (1.33%) (25), α-cadinene (1.26%) (26), caryophyllene oxide (1.24%) (6) and β-caryophyllene (27) (1.08%).



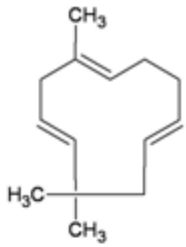
15. myrcene



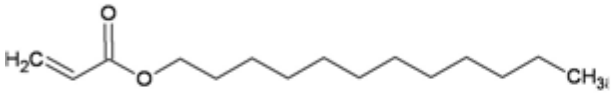
16. β-phellandrene



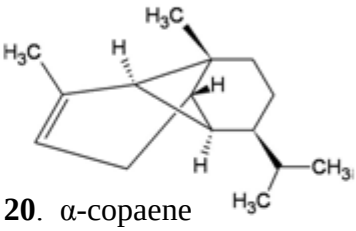
17. eugenol



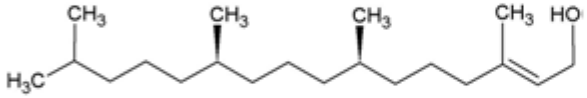
18. α-humulene



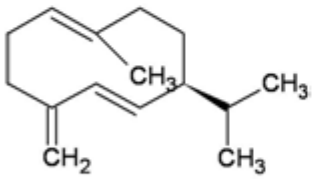
19. dodecyl acrylate



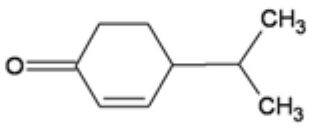
20. α-copaene



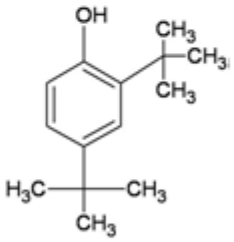
21. phytol



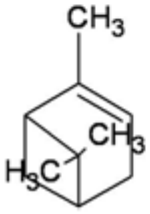
22. germacrene D



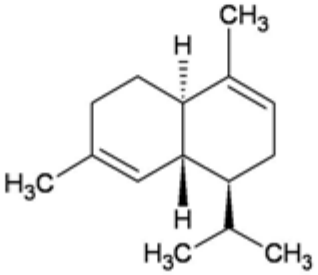
23. cryptone



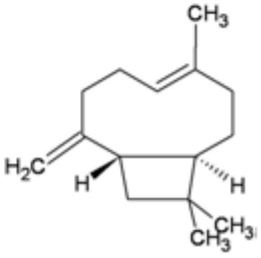
24. 2,4-ditertbutylphenol



25. α-pinene



26. α-cadinene

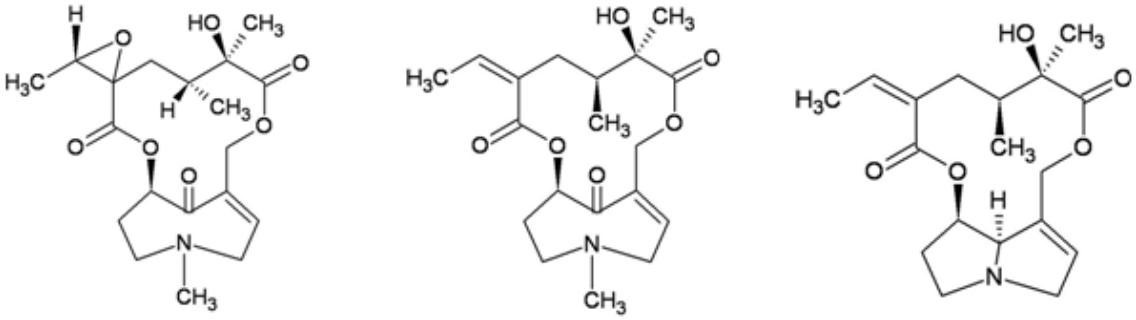


27. β-caryophyllene

Figure 1.3 Major constituents of the oil from the aerial parts of *Gynura cusimbua*

1.2.3 *Gynura sarmentosa*

Otosenine (28), senkirkine (29), and senecionine (30) (Figure 1.4) were isolated from the methanolic extract of whole aerial parts of *G. sarmentosa* (Matheson & Robins, 1992).



28. Otosenine **29**. Senkirkine **30**. Senecionine

Figure 1.4 Pyrrolizidine alkaloids of *Gynura sarmentosa*

1.2.4 *Gynura bicolor*

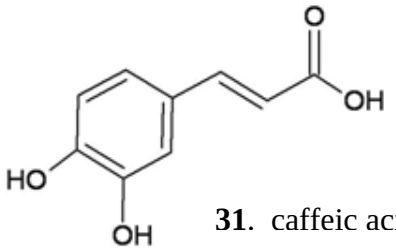
Seventeen compounds were first separated from aerial part of *G. bicolor*. They were octadecanol, undecanoic acid, hexacosanoic acid, triacontanoic acid, p-hydroxybenzoic acid, hexane, kaempferol, kaempferol-3-O-β-D-glucoside, hispidulin, kaempferol-3-O-β-D-glucopyranosyl (6→1)-α-L-rhamnoside, quercetin-3-O-β-D-glucopyranosyl (6→1)-α-L-rhamnoside, quercetin-3-O-β-glucoside, β-amyrin, α-amyrin, β-amyrin-3-O-β-glucoside, acetyl epifriedelinol, and β-sitosterol (Zhuo *et al.*, 2008).

The evaluation of hypoglycemic effect of ethanol extracts from *G. bicolor* in starch and glucose induced hypoglycemic mice revealed that the *G. bicolor* possessed some anti-hypoglycemic effect. Ethanol extract from *G. bicolor* can improve glucose tolerance in normal mice, and can decrease fasting glucose and postprandial blood glucose

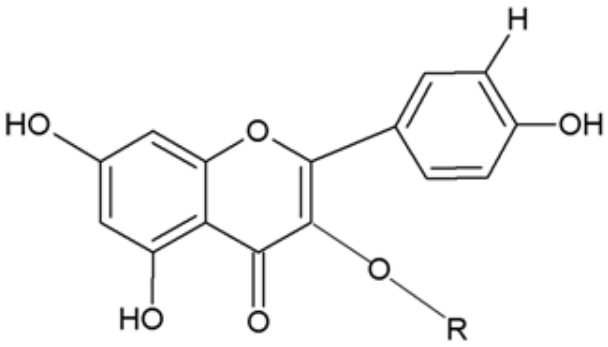
significantly. Ethanol extract from *G. bicolor* can inhibit the α-glucosidase activity, which may be the possible mechanism of hypoglycemic effect (Zheng *et al.*, 2007).

1.2.5 *Gynura formosana*

G. formosana Kiamnra (compositae) is a herbal folk medicine that is a popular vegetable in Taiwan. Four phenolics: caffeic acid (31), quercetin 3-O-rutinoside (32), kaempferol 3-O-rutinoside (33) and kaempferol 3-O-robinobioside (34) (Figure 1.5) were first isolated from *G. formosana* and the free-radical scavenging activities of a 70% aqueous acetone extract from the herb *G. formosana* were evaluated (Hou *et al.*, 2005).

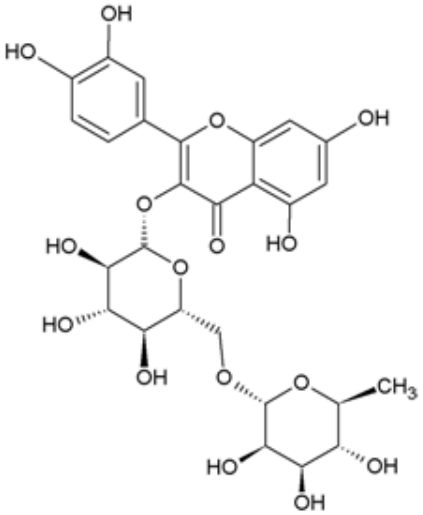


31. caffeic acid



33. kaempferol 3-O-rutinoside
(R = Rutinosyl)

34. kaempferol 3-O-robinobioside
(R = Robinobiosyl)

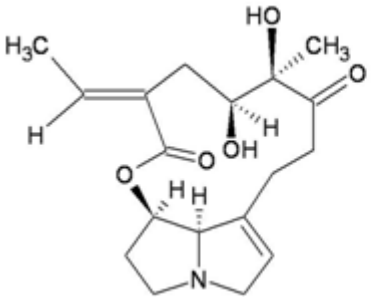


32. quercetin 3-O-rutinoside

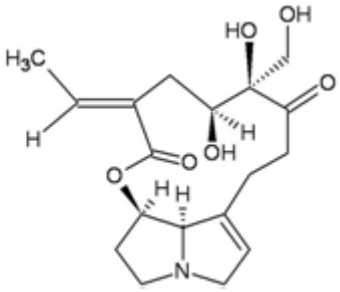
Figure 1.5 The structures of four phenolics from *Gynura formosana*

1.2.6 *Gynura divaricata*

A number of phytochemistry studies have been conducted on *G. divaricata* and at least fifty compounds have been reported from this plant. Two alkaloids, integerrimine (35) and usaramine (36), were isolated (Figure 1.6) and characterized from *G. divaricata* (Roeder *et al.*, 1996).



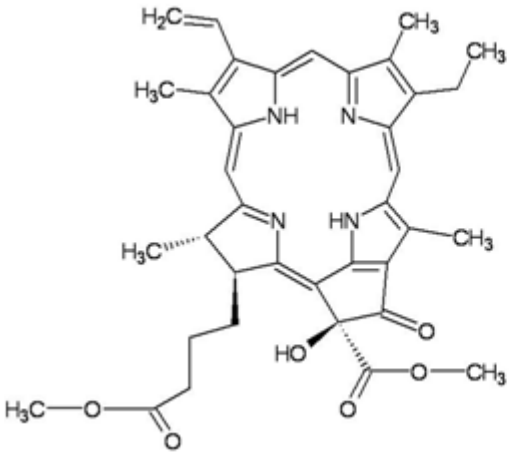
35. integerrimine



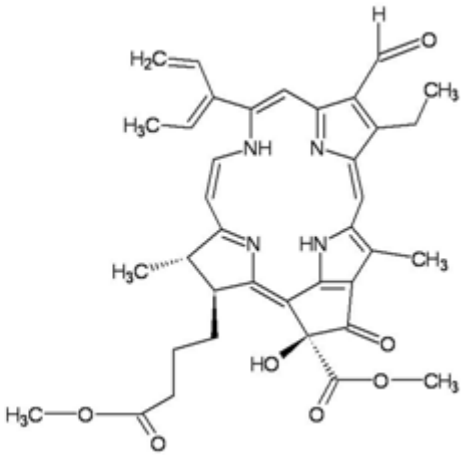
36. usaramine

Figure 1.6 Two alkaloids from *Gynura divaricata*

One alkane, two alcohols, four fatty acid esters, four triterpenes, two pheophorbides, and two mixtures of phytosterols and their glycosides were isolated from the chloroform extract of the whole herb of *G. divaricata* subsp. formosana. Among the isolates, only two pheophorbides [methyl 13-hydroxy-(13-*S*)-pheophorbide a (37), methyl 13-hydroxy-(13-*S*)- pheophorbide b (38)] exhibited potent antiproliferative activity against HL-60 cell lines (Chen *et al.*, 2003) and their structures shown in Figure 1.7.



37. methyl 13-hydroxy-(13-*S*)-pheophorbide a

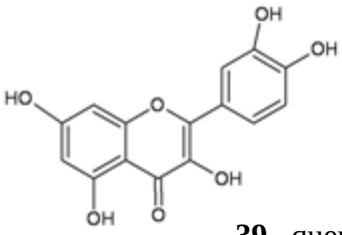


38. methyl 13-hydroxy-(13-*S*)-pheophorbide b

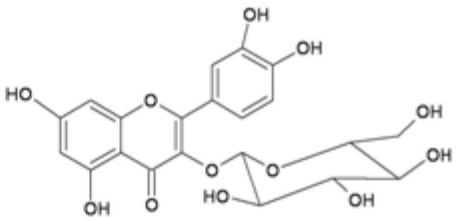
Figure 1.7 Two pheophorbides from *G. divaricata subsp. formosana*

Ten compounds (Figure 1.8) were first isolated from above ground parts of *G. divaricata* by Hu *et al*. (2006) and identified as quercetin (39), 3-O-β-D-glucopyranosyl quercetin (40), 3-O-β-D-glucopyranosyl(6→1)-α-L-rhamnosyl quercetin (41), 3-O-β-D-glucopyranosyl(6→1)-α-L-rhamnosyl kaempferol (42), epifriedelinol (43), acylated epi-friedelinol (44), β-sitosterol (45), stigmasterol (46), adenosine (47) and uridine (48).

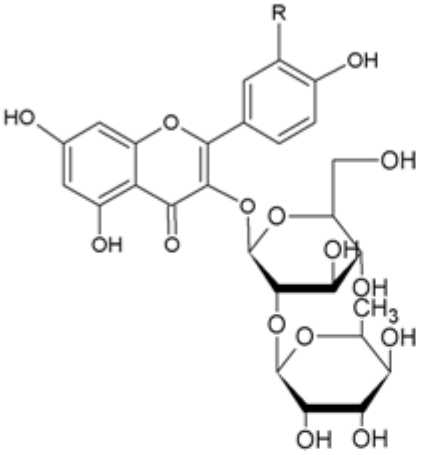
Ten compounds were isolated from *G. divaricata* by Li *et al.* (2008) and identified as follows: n-eicosane (49), tetracosanol (50), octacosanoic acid (51), octacosanol (52), palmitic acid (53), stigmasterol-3-O-β-D-glucopyranoside (54), stigmasterol (46), β-sitosterol (45), daucosterol (55) and friedelin (56) (Figure 1.9).



39. quercetin

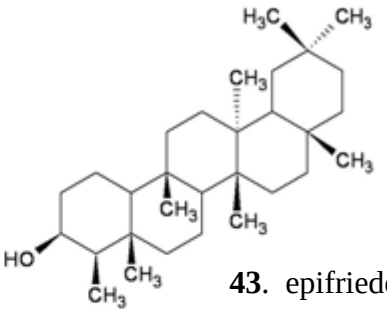


40. quercetin-3-O-β-D-glucopyranosyl

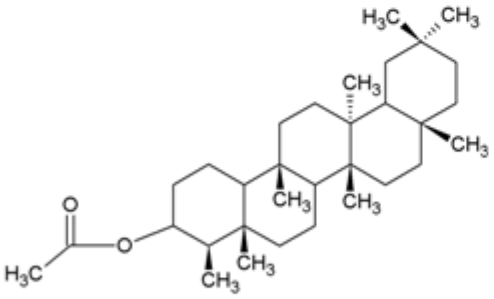


41. quercetin-3-O-β-D-glucopyranosyl(6→1)-α-L-rhamnosyl (R=OH)

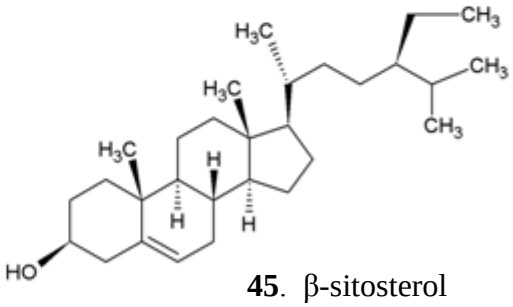
42. kaempferol-3-O-β-D-glucopyranosyl(6→1)-α-L- rhamnosyl (R=H)



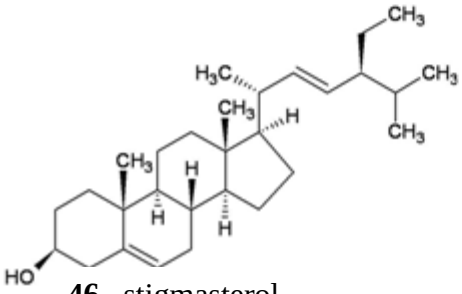
43. epifriedelinol



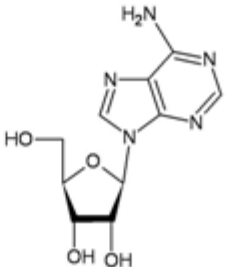
44. acylated epifriedelinol



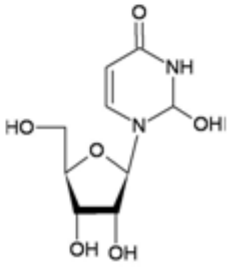
45. β-sitosterol



46. stigmasterol

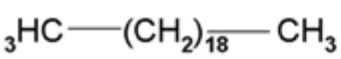


47. adenosin

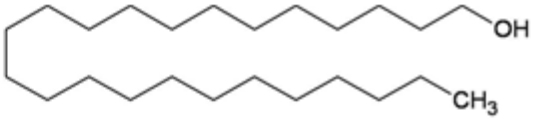


48. uridine

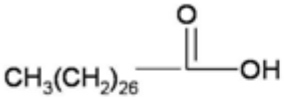
Figure 1.8 Ten compounds from above ground parts of *G. divaricata*



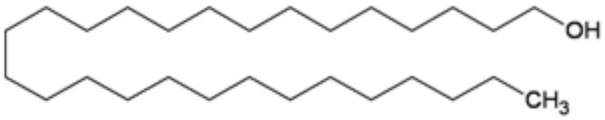
49. n-eicosane



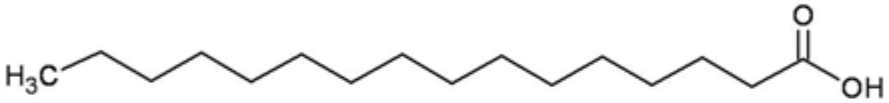
50. tetracosanol



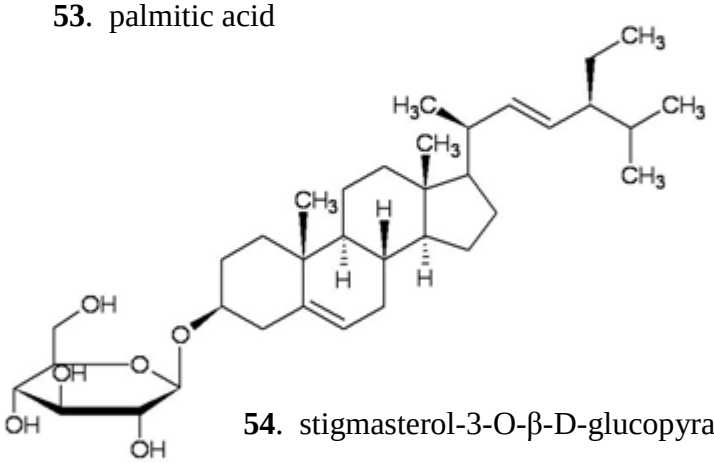
51. octacosanoic



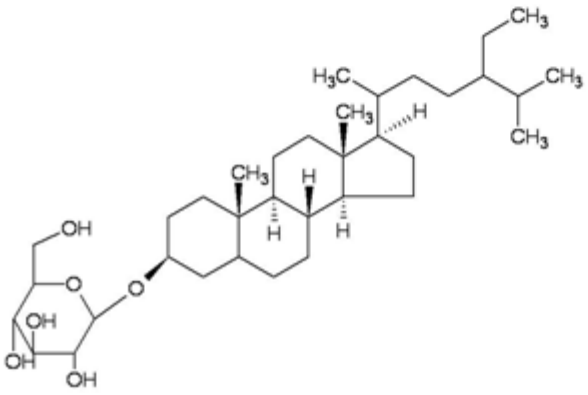
52. octacosanol



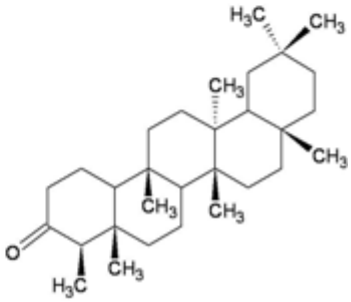
53. palmitic acid



54. stigmasterol-3-O-β-D-glucopyranoside



55. daucosterol

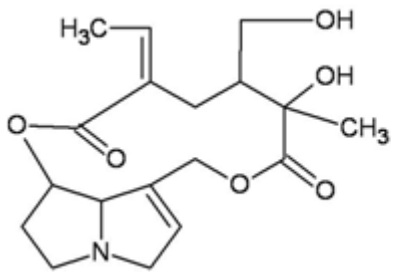


56. friedelin

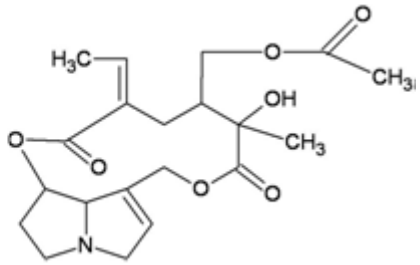
Figure 1.9 Compounds from *G. divaricata*

1.2.7 *Gynura scandens*

G. scandens yielded 2 new pyrrolizidine alkaloids, named gynuramine (57) and acetylgynuramine (58), whose structures (Figure 1.10) were detected by spectral means (Wiedenfeld, 1982).



57. gynuramine

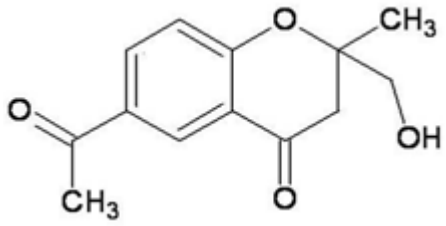


58. acetylgynuramine

Figure 1.10 Pyrrolizidine alkaloids from *Gynura scandens*

1.2.8 *Gynura elliptica*

6-acetyl-2, 2-dimethylchroman-4-one and vanillin isolated from the chloroform fraction of the roots of *G. elliptica* showed antiplatelet aggregation activity induced by arachidonic acid *in vitro* (Lin *et al.*, 2000). The structure of 6-acetyl-2, 2-dimethylchroman-4-one (59) is presented in Figure 1.11.



59. 6-acetyl-2, 2-dimethylchroman-4-one

Figure 1.11 Structure of 6-acetyl-2, 2-dimethylchroman-4-one

1.2.9 *Gynura procumbens*

The leaves of *G. procumbens* have been studied for its chemical constituents. The active chemical constituents of G. procumbens leave include, flavonoids, sterols and their glycosides (Akowuah *et al.*, 2002; Sadikun *et al.*, 1996)

A number of authors studied the hypoglycaemic activity of the leaves of *Gynura procumbens*. Zhang and Tan (2000) investigated the effects of the ethanolic extract of the leaves of *G. procumbens* on blood sugar and lipid levels in experimental animals. The extracts were found significantly suppressed the elevated serum glucose levels in diabetic rats, but did not reduce the elevated serum glucose levels in normal rats. The effects of *G. procumbens* aqueous extracts on blood glucose level and sperm quality in streptozotocin-induced male diabetic Sprague Dawley rats were investigated by Sani *et al.* (2008). They found that the administration of *G. procumbens* aqueous extract had the ability to decrease blood glucose level, restore the fertility and increase spermatogenesis in male diabetic rats. Hypoglycaemic activity of the methanol extract and n-butanol fraction of leaves of *G. procumbens* was evaluated by Akowuah *et al.* (2002; 2001). Both the methanol extract and n-butanol fraction reduced the blood sugar level in streptozotocin-induced diabetic rats. However, no hypoglycaemic effect was observed in normal rats.

The antihypertensive effects of aqueous extracts of *G. procumbens* were examined by Kim *et al.* (2006). Oral administration of *G. procumbens* extracts was found significantly lowered the blood pressure in spontaneously hypertensive rats ($P < .05$).

Iskander *et al*. (2002) reported the anti-inflammatory activity of the crude ethanolic extract of *G*. *procumbens*. The ethanolic extract, n-hexane and toluene fractions of *G*. *procumbens* were found to inhibit croton oil-induced mouse ear oederma by 65.2%, 44.6% and 34.8%, respectively.

The antioxidant potency of *G*. *procumbens* leaves extract and fractions were investigated by Yam *et al*. (2008). The total phenolic contents of the extract and fractions were detected using high performance thin layer chromatography (HPTLC). Akowuah *et al*. (2009) investigated the effect of extraction temperature on total phenolic contents and free radical scavenging. The extracts obtained at lower temperature were found to exhibit significant free radical-scavenging activity

1.3 Plant *Gynura segetum* (Lour.) Merr.

1.3.1 Plant taxonomy

Division: Spermatophyta

Subdivision: Angiospermae

Class: Dicotyledonae

Subclass: Asteridae

Order: Asterales

Family: Asteraceae/Compositae

Genus: *Gynura*

Species: *segetum*

(Suharmiati & Maryani, 2003)

Figure 1.12 Plant *Gynura segetum* (Lour.) Merr.

Figure 1.13 Flower of *Gynura segetum* (Lour.) Merr.

1.3.2 Plant morphology

The leaves are shortly petiole, ovate to ovate-lanceolate in shape, up to about 30cm long and 10cm broad. The apex is acuminate and asymmetric at the base. The leaves are fleshy with serrated margin and both surfaces are pubescent. The upper surface is green and lower surface is purplish green. Stem upright, branches profusely, purplish green in color, and slightly hairy.

The inflorescence is raceme borne on a pubescent peduncle about 20-30cm long. The flowers are hermaphrodite in nature, about 1.5cm in length, having a tubular five-toothed calyx and a yellowish orange corolla. The flowers have a strong and foetid odour. Fruits are small and brown in color. The seeds are brown and needle in shape, about 0.5cm in length.

The plants produce numerous roots which are attached to a tuber. Roots are light brown in color and usually much wrinkled. The tuber if entire is more or less conical and from 3 to 6cm long and diameter ±3cm; externally brownish grey.

1.3.3 Plant habitat

G. segetum is a cultivated species and can be found growing in the tropical from sea level up to 1200m altitude. It grows well in the highlands with flowers. While in the lower lands, the growth is relatively slower and almost never flowers. It can be seen growing in damp soil and rarely found in the wild in Malaysia.

1.3.4 Plant growth habit/cultivation

The plant is usually propagated through rhizome divisions, stem cuttings or cuttings obtained from matured mother plants. Although the plant can be grown in full sun but it does better in light shade (ideally 60% of sunlight). The leaves can be harvested when the plant is 4 months old.

1.3.5 Uses in traditional medicine

In Malaysia, the use of this plant is restricted to only the traditional healers and Malay consumed it as a vegetable (ulam). In Indonesia, the leaves and rhizome are considered to have medicinal value for both internal and external used. This herb can be combined with other medicinal plants to treat cancer, diabetes and hypertension. It is used externally for treatment of bleeding wounds, bruises, boils and sores, septic nails and ulcerous wounds. The fresh rhizome is crushed and applied externally on the wounds caused by bites of wild animal. The mixture of powdered rhizome with aloe vera is useful for treating burns and scalds. It is consumed as a vegetable (ulam) among local peoples. Leaves are consumed either raw, as salad, or cooked. Juice of leaves mix with carrot and tomato is a popular cooling drink (Suharmiati & Maryani, 2003).

In China, *G. segetum* is widely used for several applications. This plant is claimed to have anticoagulant effect used for treating snake-bite, inflammation, and other skin afflictions (Yuan *et al.*, 1990). Decoction of the roots is used to relieve heat, while decoction of the leaves is used to treat cough. Powder of *G. segetum's* dried leaves is

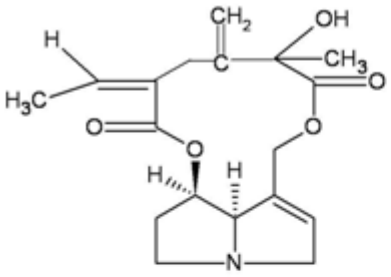
applied externally on diseased parts for early cure and it is claimed to be very effective to reduce the swelling on the body in chinese medicine.

1.3.6 Previous work on *Gynura segetum*

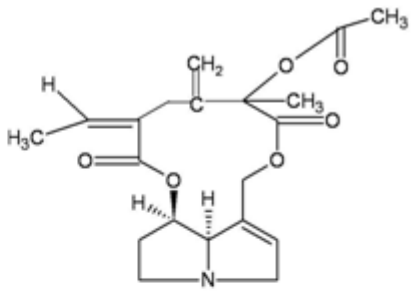
Tang *et al*. (1980) isolated a pyrrolizidine alkaloid from *G. segetum* and the compound found to be a potent antimalarial constituent. A senecionine (30) was elucidated by Hua *et al.* (1983) and also reported by Liang *et al.* (1984).

Succinic acid, D-mannitol, thymine, adenine, Ammonium chloride (NH_4Cl) and pyrrolizidine alkaloids were isolated and identified by Liu *et al.* (1988) from aerial portion of *G. segetum* plant. All of them were found to have potent anti-inflammatory activities.

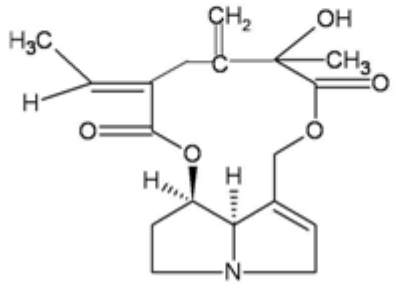
Six alkaloids were isolated by Yuan *et al.* (1990) and four of them were identified as follows: senecionine (Alkaloids I) (30), seneciphylline (Alkaloids II) (60), seneciphyllinine (Alkaloids III) (61) and (*E*)-seneciphylline (Alkaloids IV) (62). Alkaloids III and IV were found to be new compounds isolated from *G. segetum*. The structures of compounds are shown in Figure 1.14.



60. seneciphylline



61. seneciphyllinine



62. (*E*)-seneciphylline

Figure 1.14 Alkaloids from *G. segetum*

A literature survey indicates lack of research on *G. segetum* plant. Therefore, investigations of *G. segetum* are very desirable and recommended. In the present investigations, antimicrobial effect and *in vivo* antiangiogenic effect of *G. segetum* leaf extracts and fractions were carried out. The chemical compositions of the potential antimicrobial and antiangiogenic constituents in active extracts were identified by gas chromatography-mass spectrometry (GC-MS) analysis.

1.4 Antimicrobial activity

1.4.1 Introduction

In health and in disease, the activities of microorganisms greatly affect human life. Whether in country or city, tropics, mid latitudes, or arctic, human beings are continually being influenced by microbes (Thomas & Katherine, 1974).

Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century (He & Zhou, 2007). Therefore, there is a need to develop alternative anti-microbial drugs for the treatment of infectious diseases from various sources such as medicinal plants (Berahou *et al.*, 2007)

Plants have long provided mankind with herbal remedies for many infectious diseases and even today, they continue to play a major role in primary health care as therapeutic remedies in developing countries (Tshikalange *et al.*, 2005). Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Parekh & Chanda, 2007).

1.4.2 Kind of antibiotics and their actions

An antibiotic is a chemical substance produced by one microorganism that is able to kill or inhibit the growth of other microorganisms. Thousands of antibiotics have been discovered, but only a relative few have turned out to be of great practical value in

medicine. However, the most important of these antibiotics have found widespread and revolutionary use in the treatment of many infectious diseases (Thomas & Katherine, 1974)

1.4.2.1 Gentamicin

Gentamicin, a widely used aminoglycoside, is often the first drug administered for serious infections caused by gram-negative bacteria, especially those in urinary tract infections. It is also combined with other antibiotics in the treatment of diseases due to gram-positive bacteria. The antibiotic is produced by species of *Micromonospora*, a bacterium related to *Streptomyces*. Damage to the kidneys and the hearing mechanism is indicative of its toxicity (Alcamo, 1990)

1.4.2.2 Chloramphenicol

This broad-spectrum antibiotic, produced biologically by a *Streptomyces* species, was the first antibiotic also to be produced by chemical synthesis. The antibiotic inhibits protein synthesis in both gram-positive and gram-negative procaryotes. Chloramphenicol does have some toxicity in man and has caused anemia and death when used for prolonged periods at high doses, although it is relatively safe for short-term uses. It's most common medical use today is in the treatment of typhoid fever and rickettsial infections such as typhus and Rocky Mountain spotted fever (Thomas & Katherine, 1974)

1.4.2.3 Penicillin

Penicillin, the first antibiotic discovered, has been the most useful. The natural penicillins are primarily effective against gram-positive bacteria, gram-negative cocci, and the syphilis spirochete (Jensen & Wright, 1993). All penicillins have a similar mode of action, affecting cell-wall synthesis in procaryotes. Penicillins do not inhibit growth of eucaryotic cells and are therefore quite nontoxic to man and most animals. However, penicillin does cause allergic reactions in some people and hence are not completely harmless (Thomas & Katherine, 1974)

1.4.2.4 Tetracycline

The tetracyclines are broad-spectrum antibiotics and are useful against a wide variety of gram-positive and gram-negative bacteria, although in contrast to streptomycin they are not useful in the treatment of tuberculosis. Despite their lack of human toxicity, the tetracyclines must still be administered cautiously, since when given orally they alter the normal bacteria of the intestine and may cause intestinal disturbances (Thomas & Katherine, 1974)

1.4.2.5 Ampicillin

Ampicillin exemplifies a semisynthetic penicillin. It is less active against gram-positive cocci than penicillin G, but is valuable against several gram-negative rods as well as gonococci and meningococci. The drug resists stomach acid and is absorbed from the intestine after oral consumption (Alcamo, 1990).

1.4.2.6 Amphotericin B

Amphotericin B, produced by *Streptomyces nodosus*, is a widely used antibiotic for treatment of very severe internal infections caused by certain pathogenic fungi (Pelczar *et al.*, 1993). However, it causes a wide variety of side effects and therefore is used only in progressive and potentially fatal cases (Alcamo, 1990).

1.4.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing can provide useful information to physicians in the selection of appropriate antimicrobial therapy for patient care. It is an effective means of improving antibiotic use. Antimicrobial susceptibility testing can be carried out by different techniques. The agar diffusion method can offer a category result based on a zone size. Broth and agar dilution method can be used to determine a minimum inhibitory concentration value of an antibiotic (He & Zhou, 2007).

The agar diffusion method operates on the principle that antibiotics will diffuse from a paper disc or small cylinder into an agar medium containing test organisms (Alcamo, 1990). In the agar diffusion method, the microbiologist swabs an agar plate with the test organism and then places small paper disc impregnated with known amounts of antibiotic upon the surface of the agar. After incubation, the plates are observed for clear zones around the discs. A clear zone indicated that the antibiotic which has diffused into the agar from the disc, has prevented the organism from growing. The absence of a clear zone means that the organism is resistant to the antibiotic (Pelczar *et al.*, 1993).