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ANTIMICROBIAL EFFICACY, ANTIOXIDANT PROFILE AND NINE ALTERNATIVE ACTIVE CONSTITUENTS FROM PETROLEUM ETHER AND ETHYL ACETATE EXTRACT OF *Entada spiralis*

(Keberkesanan Antimikrob, Profil Antioksida dan Sembilan Komponen Alternatif Aktif dari Ekstrak Petroleum Eter dan Etil Asetat dari Pokok *Entada spiralis*)

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Abstract

This study aimed to investigate the antimicrobial activity of petroleum ether extracts and the identification of alternative antimicrobial and antioxidative constituents from petroleum ether extract and ethyl acetate extract of *Entada spiralis* stem. Antimicrobial activity was evaluated through disc diffusion method on five human superficial skin disease-caused microbes such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trychophyton tonsurans* and one plant pathogen namely *Erwinia chrysanthemi*. The presence of antioxidants was determined from thin layer chromatography (TLC) sprayed with 2,2-diphenyl-1-picrylhydrazyl. The isolation of antioxidant and antimicrobial compounds was performed through preparative TLC. The structure of isolated compounds was determined from gas chromatography mass spectrometry (GCMS) and liquid chromatography mass spectrometry (LCMS) equipped with Wiley Library matching individually. The petroleum ether extract successfully inhibited the growth of all bacteria and dermatophytes in a concentration dependent manner whereby *S. epidermis* was highly susceptible towards the extract with inhibition zone of 16.0 mm at concentration of 400 mg/mL as well as *M. gypseum*. Most of the components from petroleum ether extract and ethyl acetate extract developed on TLC were antioxidative which was seen as yellow spots against purple background after spraying with DPPH reagent. Four antioxidative constituents were successfully isolated and tentatively identified as 18,19-Secoyohimban-19-oic acid,16,17,20,21-tetradehydro-16-(hydroxymethyl)-methylester **(1**), Oxiraneoctanoic acid **(2),** 9,12-Octadecadienoic acid **(3),** and 11-O-p- Coumarylnepeticin **(4)**. Five antimicrobial constituents were successfully isolated and tentatively identified as 4-benzyloxy-4-[2,2,-dimethyl-4-dioxolanyl]Butylaldehyde, **(5),** Isobutyl octadecyl benzoate ester **(6),** 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone **(7)**, 1,2,4,5-tetramethylbenzene **(8)** and Hordatine B **(9)**. Thus, *E. spiralis* is seen to be a promising source of bioactive ingredients which is very important as the basis of scientific information for the development of natural antimicrobial and antioxidant agents.

Keywords: Leguminoceae, *Entada spiralis*, antioxidant, antibacterial activity

Abstrak

Kajian ini memfokuskan kepada kajian sifat antimikrob ekstrak petroleum eter dan pencarian komponen aktif antimikrob dan antioksida dari ekstrak petroleum eter dan etil asetat dari batang pokok *Entada spiralis*. Aktiviti antimikrob dinilai menggunakan kaedah penyerakan cakera terhadap lima jenis mikrob penyakit kulit iaitu *Staphylococcus aureus*, *Staphylococcus epidermis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trychophyton tonsurans* dan satu jenis mikrob tumbuhan iaitu *Erwinia chrysanthemi*. Ciri antioksidan ekstrak komponen yang telah dipisahkan di atas kromatografi lapisan nipis (KLN) telah dianalisa menggunakan kaedah penyemburan dengan bahan 2,2-difenil-1-pikrilhidrazil (DPPH) di atas KLN. Pemisahan komponen aktif antioksida dan antimikrob telah dijalankan dengan menggunakan KLN. Spektrometer jisim kromatografi gas dan spektrometer jisim kromatografi cecair dengan data Wiley telah digunakan untuk menganalisa dan mengenalpasti struktur bahan aktif. Ekstrak petroleum eter berkesan dalam menyekat pertumbuhan bakteria dan kulat di mana bakteria *S. epidermis* dan kulat *M. gypseum* paling kuat disekat pertumbuhannya oleh ekstrak dengan zon perencatan 16.0 mm pada kepekatan 400 mg/mL. Kebanyakan komponen dalam ekstrak petroleum eter dan etil asetat yg telah dipisahkan di atas KLN bersifat antioksida dengan memberikan kesan tompokan kuning setelah disembur dengan larutan DPPH. Sebanyak empat komponen antioksida berjaya dipisahkan dan dikenalpasti sebagai Asid 18,19-Sekoyohimban-19,16,17,20,21-tetradihidro-16-(hidroksimetil)-metilester **(1**), Asid okzairanoktanoik **(2),** Asid 9,12-Oktadekadienoik **(3),** dan 11-O-p- Koumarilnepetisin **(4).** Sebanyak lima jenis komponen antimikrob berjaya dipisahkan dan dikenali sebagai, 4-benziloksi-4-[2,2,-dimetil-4-dioksolanil]Butilaldehid**(5),** Isobutil oktadesil benzoat ester**(6),** 3',8,8'-Trimetoksi-3-piperidil-2,2'-binaftalin-1,1',4,4'-tetron **(7)**, 1,2,4,5-tetramethylbenzene **(8)** dan Hordatin B **(9)**. Maka, *E. spiralis* dilihat sebagai pokok yang berpotensi untuk mendapatkan bahan bioaktif semulajadi di mana bahan ini penting sebagai maklumat asas bagi kajian penghasilan bahan antioksida dan antimikrob semulajadi.

Kata kunci: Leguminosae, *Entada spiralis*, antioksida, aktiviti antibakteria

Introduction

Free radical which is usually known as a disease source can possibly come into contact with our body either directly or indirectly by damaging internal cell *via* oxidation process and this may cause several diseases. Thus, to combat free radicals, an antioxidant is a good alternative to consume externally or internally. It is a molecule that restrains the oxidation of other molecules. Generally, oxidation is a chemical reaction that can produce free radicals which lead to chain reactions that could destroy cells. Antioxidants such as thiols or ascorbic acid (vitamin C) stop these chain reactions by preventing some degeneration of certain diseases such as cancer by inhibiting the reaction of radical. Antioxidant of phytochemicals from plant such as flavonoids and terpenoids are able to block the reaction of free radical and in the same way, protect human body from suffering due to a disease [1]. The antifungal efficacy of *E. spiralis* against human pathogens has been well-documented [2] in which methanol extract was declared as the most active extract as well as its fractions [3]. The antibacterial activity of stem and leaves of *E. spiralis* against *E. chrysanthemi* has recently been reported [4] in which ethyl acetate extract was claimed as the most active extract.

The isolation and structure elucidation of compounds from seed kernels of *E. rheedii* showed the presence of two triterpenoid saponins [5] but unfortunately, they were not antioxidative. For the past few years, the antibacterial and antidermatophytic constituents from methanol extracts of *E. spiralis* have previously been isolated and identified [6, 7, 8], however, there was lack of identification of antioxidative and antimicrobial compounds from other *E. spiralis* extracts. Currently, antioxidative constituents from methanol extract of the stem bark of *E. spiralis* have been successfully determined and they were reported as tetrahydroxyflavone [9], ferulic ester such as (E)-hexyl 3-(4-hydroxy-3-methoxyphenyl) acrylate and five known phenolic constituents identified as kaempferol, 5,4'-dihydroxy-3,7,3'-trimethoxyflavone, gallic acid, (+)-catechin, as well as (-)-epicatechin, with gallic acid being the most antioxidative [10].

Therefore, we embarked our investigation upon antimicrobial activity, antioxidant profile and

identification of alternative antioxidative and antimicrobial compounds from petroleum ether and ethyl acetate extracts from *E. spiralis* stem.

Material and Methods

Sample preparation

About 1 kg stem of *E. spiralis* was obtained from Pahang's Tasik Chini forest and authenticated by a botanist with voucher specimen given as KMS-2558. It was ground into powdered form and soaked for three consecutive times with 3L of petroleum ether, dichloromethane and methanol. The maceration was filtered and evaporated under vacuum to obtain crude extracts. All extracts were kept in refrigerator before use.

Fourier transform infrared (FTIR) analysis

A small amount of stem extract was mixed with ethanol. Then, it was placed onto sample detector of Attenuated Total Reflectance FTIR (ATR-FTIR) Perkin-Elmer SPECTRUM 100 FTIR to generate an infrared spectrum. The interpretation of infrared spectrum was conducted by means of determining functional groups in the stem extract of *E. spiralis.*

Preparation of spraying reagents for thin layer chromatography (TLC) screening: $FeCl_3$ reagent

An amount 1 g of ferric chloride was dissolved in mixture of 50 mL of methanol and 50 mL of distilled water. Dark blue spot appearing on TLC sprayed with $FeCl_3$ solution was the indication of the presence of phenolic compound.

Vanillin/ H_2SO_4 reagent

An amount 1.5 mL of concentrated sulfuric acid was added to a mixture of 1% vanillin in absolute ethanol. The grey, pink, blue and purple spots appearing on TLC which was sprayed and heated on hot plate was the indication of terpenoid compounds.

Dragendorff's reagent

Solution A: 0.85 g basic bismuth nitrate was added in mixture of 100 mL acetic acid and 40 mL of water. Solution B: 8g of potassium iodide was mixed with 20 mL of water. The spraying reagent was prepared by mixing 5 mL of solution A and 5 mL of solution B with a mixture of 20 mL of acetic acid and 70 mL of water. Orange spot against yellow background appearing on the sprayed TLC was the indication of alkaloid compound.

DPPH reagent

This reagent was prepared by mixing suitable amount of DPPH solid in methanol to obtain DPPH solution with concentration of 0.6% (w/v). The yellow spot against purple background that appeared on TLC of extracts sprayed with DPPH revealed the presence of antioxidative compounds.

TLC screening for phytochemicals

The petroleum crude extract was applied on a TLC plate as 1 cm fine bands with capillary tube. Then, the TLC plate was developed with suitable developing solvent in the chromatographic chamber until solvent front is reached. The plate was taken out and was dried for a few minutes. After completely dried, the plate was sprayed with chemical reagents. For terpenoid screening, the plate was heated directly on hot plate just after spraying with vanillin/H_2SO_4 reagent to visualize the terpenoid spots. The TLC screening process was repeated with other reagents such as $FeCl_3$, Dragendorff's and DPPH reagent to screen phenolic, alkaloid and antioxidative spots respectively.

Isolation of active compounds from preparative thin layer chromatography (pTLC)

The stem extract was applied on 10 cm x 10 cm TLC plate and was subjected to chromatographic separation. The bands of active components were marked, and desired bands were scraped off. The isolated compound was separated from silica gel through filtration and then was re-subjected to thin layer chromatographic separation to screen the purity of active components.

Gas chromatography (GC-MS)

The GC-MS analysis of isolated constituents was performed using Agilent Technologies GCMS 5977A MSD. The isolated compounds from petroleum ether extract were introduced using splitless injection of 1.0 µL in hexane fitted with cross linked of 5% phenyl methyl siloxane capillary column. The oven temperature

was at 60 - 290 °C with the rate of 5 °C min^{-1}. The injector temperature was about 220 °C.

Liquid chromatography mass spectrometer (LCMS)
The LCMS spectrum of isolated compounds from ethyl acetate extract were obtained using Vion IMS QTOF MS. Chromatographic separation was performed using Column Temperature at 40°C. The mobile phase for solvent A contained water and 0.1% formic acid, whereas for solvent B, it contained acetonitrile. The spectral data were collected at 254 nm. The high mass analysis performed was at 1000 m/z and low mass at 50 m/z under positive ion mode. The capillary voltage was set at 3.0 kV using a desolvation gas at 800 L/h. The voltage offset was at 0 mV and the full-scale voltage was at 2000 mV.

***In vitro* antimicrobial activity assay of petroleum ether extract**
The antifungal activity and antibacterial activity assay of petroleum ether extract were evaluated through disc diffusion method as previously reported [2, 3]. In antibacterial assay, the disc diffusion method was repeated in the same manner as in antifungal assay, whereby extract impregnated discs were aseptically transferred on the inoculated agar plate of muller hinton agar (MHA) and incubated for 24 hours.

Results and Discussion

FTIR analysis
FTIR spectra of petroleum ether extract which depicted in Figure 1 is used to determine types of functional groups present in petroleum ether extract. The figure reveals absorption peak at 2917.63 cm^{-1} which indicated the sp^2 C-H stretching vibration and absorption peak of sp^3 C-H stretching at 2850.16 cm^{-1}. The visibility of C=C stretching vibration was traced at absorption peak 1462.33 cm^{-1} and the adsorption peak at 1709.49 cm^{-1} was for carbonyl group, C=O stretching. The absorption peak at 1364.80 cm^{-1} belonged to absorption peak of N-H bending and the C-O functional group was determined at absorption peak of 1121.65 cm^{-1}. Since the petroleum ether extract contains C=O,C-O, C=C, C-H and N-H functional groups, we suggest that such constituents like alkaloid, ester derivatives and carboxylic acid derivatives are probably present in petroleum ether extract.

The types of functional groups present in ethyl acetate extract is illustrated in Figure 2 in which the presence of C=O stretching vibration was observed at 1709.49 cm^{-1}, C-O stretching at 1165.07 cm^{-1}, C-N stretching at 1242.86 cm^{-1} and sp^3 C-H stretching at 2917.65 cm^{-1}. The O-H stretching was found at 3240.45 cm^{-1} and the absorption peak at 1606.13 cm^{-1} and 1459.40 cm^{-1} belonged to C=C aromatic. In both extracts, all the functional groups provided basic information to determine the structure of the compounds. As far as we are concerned, some compounds bearing OH and N-H groups are able to display antioxidant behavior [9] and compounds with aromatic ring and COOH linkage have revealed remarkable antimicrobial properties[11].

TLC analysis of petroleum ether extract and ethyl acetate extract
The spots on TLC were visualized under UV short wave (TLC a), long wave (TLC b) and also sprayed with certain chemical reagents (TLC c, d, e). According to Figure 3, TLC a and b illustrated that certain spots demonstrated aromatic character since black spots on TLC a and fluorescence spot on TLC b were visualized. Thus, we suggest that the structure of compounds from desired spots may contain benzenoid ring. TLC **c** indicated an obvious spot of an alkaloid (dark orange) after being sprayed with Dragendorff's reagent, and it displayed its antioxidant properties since the spot was nearly in the same R_f with one of antioxidative spots from TLC d. Referring to TLC d, most of the compounds were antioxidative which were signaled by yellowish spot against purple background when sprayed with DPPH reagent.

Generally, the mechanism was based on the reduction of DPPH, a stable nitrogen-cantered and violet-coloured free radical, that upon reduction, was converted to yellow colour by electron or hydrogen donating ability of the antioxidant compound found in the extract [9]. The degree of discoloration indicated the scavenging potential of antioxidant compounds of extract in terms of hydrogen ability. The more the decolonization of purple colour, the greater the reduction ability was. TLC e illustrated various kinds of terpenoid compounds

(colour range of blue, purple, pink) and several of them were also antioxidative. Thus, the results of TLC analysis suggested that petroleum ether extract was antioxidative which was caused from the presence of alkaloid and terpenoid.

TLC screening method using DPPH spraying reagents is a convenient and the simplest way to detect antioxidant in extract sample because only a small amount of extract and reagent are needed, and it reveals a fast result. As depicted in Table 1, the antioxidant behaviour of ethyl acetate extract was caused by the emergence of terpenoid, alkaloid and phenolic compounds while in petroleum ether extract, only terpenoid and alkaloid were responsible for its antioxidant properties. The antioxidant properties of *E. spiralis* from methanol extract has been previously reported [10] but there is still no report of antioxidants being recorded from petroleum ether and ethyl acetate extract. Thus, this is the first report of antioxidant screening for petroleum ether extract and ethyl acetate extract from *E. spiralis* stem as well as the antimicrobial activity.

Antioxidative constituents from petroleum ether extract and ethyl acetate extract of *E. spiralis* stem

Figure 4 demonstrated three antioxidative constituents from petroleum ether extract labelled as **(1), (2)** and **(3)** and one constituent labelled as **(4)** from ethyl acetate extract of *E spiralis* stem. The structures of antioxidative constituents from petroleum ether extract were identified via GCMS with the assistance of Wiley Library matching individually and LCMS with Library Data was utilized for structure identification of constituent from ethyl acetate extract. Generally, the presence of functional groups in the structures are consistent with FTIR analysis of petroleum ether and ethyl acetate extract. FTIR analysis of petroleum ether extract has recorded the existence of C=O group, C=C for aromatic ring and nitrogen linkage. These data agreed with compound **(1)** as the structure showed the functional group of C=O, C=C, C-N and N-H linkage. Compound **(2)** and **(3)** revealed the appearance of C=O, C-O and C=C group which are still in the range of FTIR analysis data. The structure of compound **(4)** revealed the presence of OH, C=O group, C=C, C-O linkage and aromatic linkage and they were consistent with FTIR analysis of ethyl acetate extract.

According to all antioxidative structures, the hydroxyl group OH or NH group possibly functions as the main part for antioxidant effect towards DPPH radical. Theoretically, hydrogen atom from OH or NH group reacts with free radical species of DPPH through Hydrogen Atom Transfer (HAT) or Single Electron Transfer (SET). During radical scavenging process, the electron of hydrogen from NH or OH group is donated to nitrogen radical of DPPH which contains single electron through reduction reaction, which can be seen from the reduction of dark purple colour to yellow colour of DPPH [12]. After reduction process completes, the radical nitrogen has no more single electron as it has bonded with hydrogen from OH group.

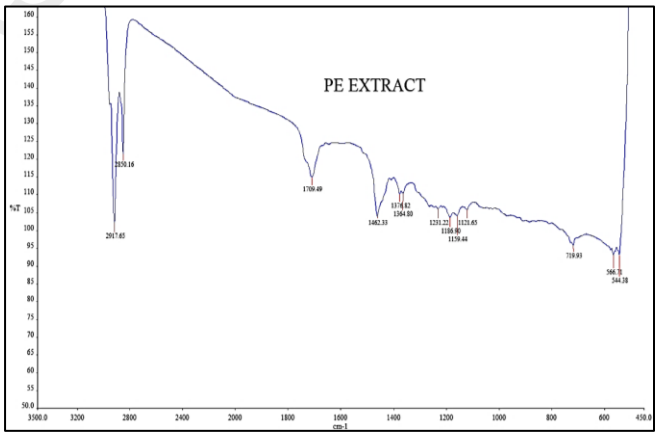


Figure 1. FTIR spectra of petroleum ether extract of *E. spiralis*

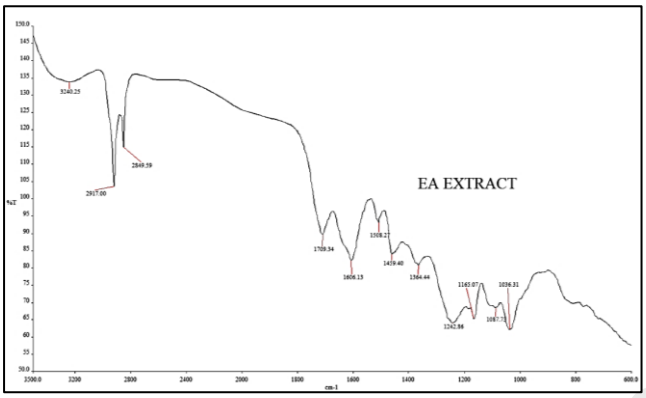
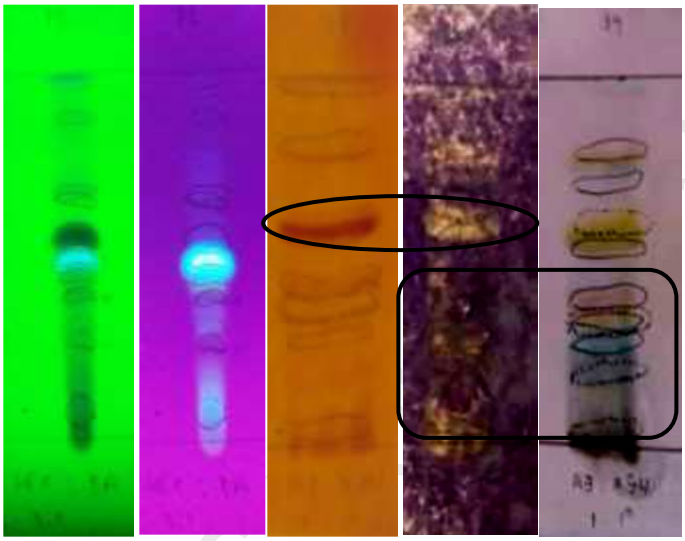


Figure 2. FTIR spectra of ethyl acetate extract of *E. spiralis*



a b c d e

Antioxidative spots (TLC d) is an alkaloid (TLC c)

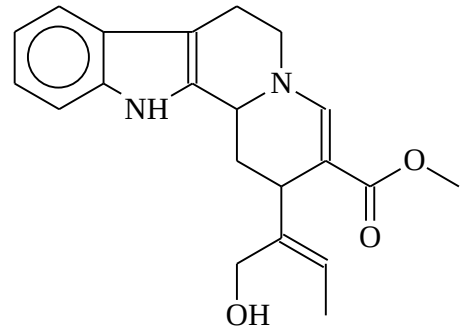
Antioxidative spots (TLC d) are terpenoids (TLC e)

Figure 3. TLC analysis of petroleum ether extract (a) UV_{254} (b) UV_{366} (c) After spraying with Dragendorff's reagent to visualize orange spot of alkaloid (d) After spraying with 2, 2 – Diphenyl – 1 – picryl – hydrazyl (DPPH) solution to visualize yellowish antioxidative spots against purple background and (e) After sprayed with Vanilin/ H_2SO_4 reagent and heated at 110^oC to visualize terpenoid spots

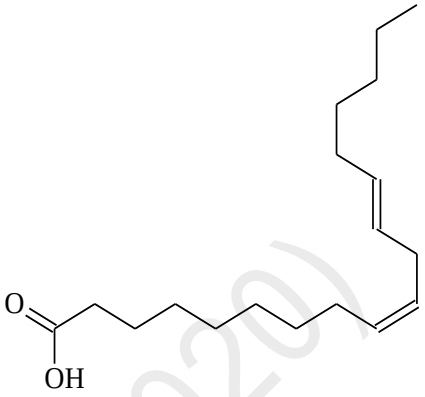
Table 1. Selected phytochemicals screened from petroleum ether and ethyl acetate extract

Extract	**Terpenoid**	**alkaloid**	**phenolic**
Petroleum ether	√*	√*	x
Ethyl acetate	√*	√*	√*

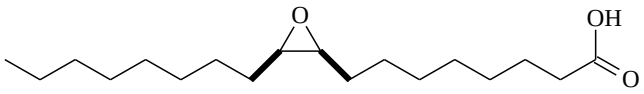
* Antioxidative ; √ = present; x = absent



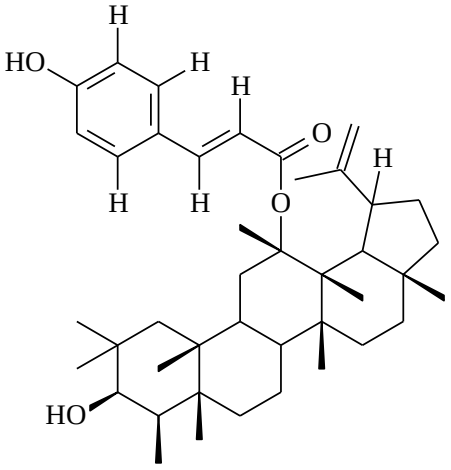
18,19-Secoyohimban-19-oic acid,16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester **(1)**



Oxiraneoctanoic acid **(2)**



9,12-Octadecadienoic acid **(3)**



11-O-p- Coumarylnepeticin **(4)**

Figure 4. Antioxidative compounds from pet ether extract and ethyl acetate extract of *E. spiralis* stem

***In vitro* Antimicrobial activity of petroleum ether extract of *E. spiralis* stem**

Table 2 and Table 3 depicted the result of antibacterial and antifungal activity of petroleum ether extracts against *S. aureus*, *S. epidermidis*, *T. tonsurans*, *T. mentagrophytes* and *M. gypseum* strains by means of the size of inhibition zone. In Kirby-Bauer disc diffusion method, the size of inhibition zone measures the compound's effectiveness in which the greater the zone of inhibition is, the more effective the compound becomes. The petroleum ether extract was strongly inhibited the growth of *S. epidermidis* with inhibition zone of 16 mm compared to *S.aureus*. Among all bacteria, *S. epidermidis* (ATCC 12228) was found to be the one that is most susceptible toward petroleum ether extract, whereas *S. epidermidis* (ATCC 13518) was found to be resistant towards petroleum ether extract. All bacteria were sensitive towards positive control ampicillin.

The petroleum ether extract of *E. spiralis* stem also inhibited the growth of all dermatophytes in a concentration dependent manner as presented in Table 2. The extract strongly inhibited the growth of *M.*

gypseum with inhibition zone of 16 mm at 400 g/mL. Thus, *M.gypseum* was recognized as the one that is most susceptible to petroleum ether extract compared to other dermatophytes. The results obtained could be generally explained in terms of the effect of the active principles in the extracts once they get into contact with cell wall of microconidia of dermatophytes. As a result, the microconidia lost its rigidity and became ruined and the growth of microconidia was no longer sustained. According to previous study [10], the sensitivity of all dermatophytes towards extracts could be due to the synergistic action of different group of active constituents comprised in the extracts, such as benzenoid compounds. These active constituents may interact with each other to enhance the antimicrobial activity of the extracts. Therefore, this action could possibly be one of the factors for our present results of antimicrobial activity of *E. spiralis* stem.

The inhibition effect in antibacterial activity was also most probably due to the interference by active sources existed in the extracts, which lead to the disruption of cell wall of bacteria and as a result, the cell wall lost its rigidity and caused cell death and consequently the growth of bacteria was no longer emerged.

Figure 5 illustrated the structures of five antimicrobial constituents from petroleum ether extract and ethyl acetate extracts of *E.spiralis* stem that may involve in inhibiting the growth of bacteria and dermatophytes. Compound **(5),(6), (7)** and **(8)** were identified from petroleum ether extract and compound **(9)** was identified from ethyl acetate extract. We had previously reported the data of antibacterial effect of ethyl acetate extract against plant pathogen [4]. Compounds **(5), (6), (7)** and **(8)** can be regarded as new antimicrobial constituents since no investigation to identify antimicrobial constituents from petroleum ether extract of *E. spiralis* has been reported. Meanwhile, compound **(9)** Hordatine B is not a new compound in other plant species, but it can be suggested as a new antimicrobial compound since it was identified for the first time from ethyl acetate of *E. spiralis* stem.

Theoretically, the action of antimicrobial activity starts when the constituent attacks the cell wall of bacteria to prevent them from keeping on synthesizing a peptidoglycan; a molecule that provides the strength of cell wall in order to survive in human body. Attacking cell wall may disrupt membrane potential through depolarization and this leads to the death of cell wall of bacteria. Antifungal agent works by affecting a substance in the cell membrane of fungi, causing the contents of the fungal cells to leak out and the cells to die, thus preventing the fungal cells from growing and reproducing. The antifungal agent such as azoles is a five-membered heterocylic compound containing nitrogen (N) atom. Since structure **(7)** and **(9)** are also compound containing nitrogen atom, it is possible for the structures to have same the function as antifungal agent just like azoles. Another antimicrobial agent is Echinocandin B [13]. The C=O(NH) linkage in Hordatine B **(9)** is quite consistent with the C=O(NH) linkage in Echinocandin B and benzene-OH group linkage. Thus, this similarity may explain the antimicrobial properties of **(9).**

Table 2. *In vitro* antibacterial activity of petroleum ether extract of *E. spiralis* stem

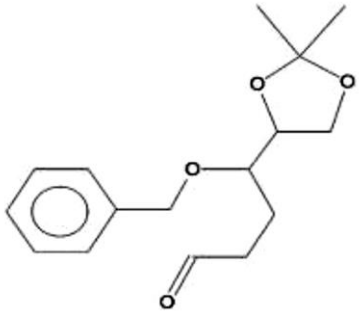
Extracts Concentration (mg/mL)		**Inhibition Zone (mm)[a]**			
		SA 33591	**SA 33592**	**SE 12228**	**SE 13518**
Petroleum ether extract	50	14.0 ± 0.71	14.50 ± 0.71	12.0 ± 0	-
	100	15.0 ± 0	15.0 ± 0	15.0 ± 0	-
	200	15.0 ± 0	15.0 ± 0	15.5 ± 0	-
	400	15.0 ± 0	15.33±0.58	16.0 ± 1.61	-
Standard Ampicillin		9.0 ± 0	10.0 ± 0	19.0 ± 1.0	23.0 ± 1.4

- No activity; SA, *Staphylococcus aureus*; SE, *Staphylococcus epidermidis*; ±, Standard deviation (S.D); [a] Mean of three replicates. Statistical significance was determined using ANNOVA. Differences were considered significant at (p <0.05). 16 mm above: highly inhibited; 11-15 mm: moderately inhibited; 6-10 mm: weakly inhibited.

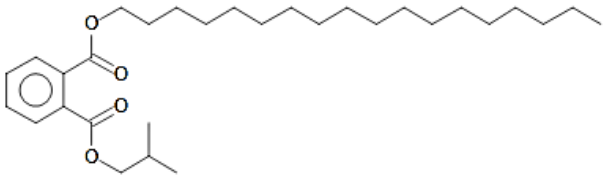
Table 3. *In vitro* antifungal activity of petroleum ether extract of *E. spiralis* stem

Extracts Concentration (mg/mL)		**Inhibition Zone (mm)[a]**		
		TM	**TT**	**MG**
Petroleum ether extract	50	-	8.67±0.58	9.50±0.71
	100	8.33±0.58	10.3±0.58	10.0±0
	200	9.0±0.00	9.00±1.0	13.0±0
	400	10.67±0.58	13.0±1.73	16.0±0
Standard Nystatin		21.33±0.58	40.33±0.58	24.0±0

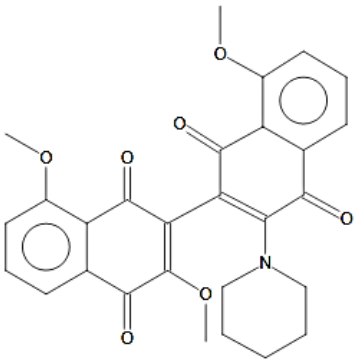
- No activity; TM, *Trichophyton mentagrophytes*; TT, *Trichophyton tonsurans*; MG, *Microsporum gypseum*; CG, *Candida glabrata*; ±, Standard deviation (S.D); [a] Mean of three replicates. Statistical significance was determined using ANNOVA. Differences were considered significant at (p <0.05). 16 mm above: highly inhibited; 11-15 mm: moderately inhibited; 6-10 mm: weakly inhibited



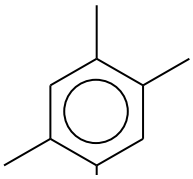
4-benzyloxy-4-[2,2,-dimethyl-4-dioxolanyl] Butylaldehyde **(5)**



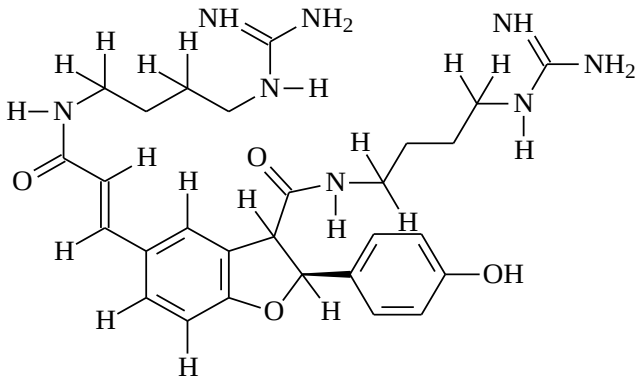
Isobutyl octadecyl benzoate ester **(6)**



3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone **(7)**



1,2,4,5-tetramethylbenzene **(8)**



Hordatine B **(9)**

Figure 5. Antimicrobial active constituents from petroleum ether extract of *E. spiralis* ste

Conclusion

The petroleum ether extract and ethyl acetate extract from stem of *E. spiralis* displayed antioxidant properties and potent antimicrobial activity. Four antioxidative constituents were successfully isolated and tentatively recognized as 18,19-Secoyohimban-19-oic acid,16,17,20,21-tetradehydro-16-(hydroxymethyl)-methylester **(1**), Oxiraneoctanoic acid **(2),** 9,12-Octadecadienoic acid **(3),** and 11-O-p-Coumarylnepeticin **(4)**. Five antimicrobial constituents were isolated and tentatively identified as 4-benzyloxy-4-[2,2,-dimethyl-4-dioxolanyl]Butylaldehyde, **(5),** Isobutyl octadecyl benzoate ester **(6),** 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone **(7)**, 1,2,4,5-tetramethylbenzene **(8)** and Hordatine B **(9).** All active constituents may be regarded as new compounds since no investigation from petroleum ether extract and acetate extract of *E. spiralis* were used and reported. Thus, this plant appears to be a good source of antimicrobial and antioxidant agents as well. However, further spectroscopic analysis study such as 1D and 2D NMR need to be carried out for structure confirmation.

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