Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.3, 2014

Study on anti-quorum sensing potentials and phytochemical constituents of *Euphorbia hirta*

*Nur Aishah Abdul Wahab, Hanina Mohd Noor, Salina Mat Radzi and Jalil Kader
Faculty of Science and Technology, Universiti Sains Islam Malaysia
71800 Nilai Negeri Sembilan, Malaysia
Tel: +6014-8409085
*E-mail of correspondence author: nuraishahabdulwahab@gmail.com.my

The research is financed by USIM Administrator Grant Research.

ABSTRACT

Euphorbia hirta is an annual broad-leaved herb and widely used as traditional medicine to treat various ailments. This herb was tested on the anti quorum sensing (anti-QS) potentials in fresh (edible or macerated) forms and acetone extracts *via* biomonitor strain *Chromabacterium violaceum* (ATCC 12472). The biomonitor strain has an ability to produce purple pigment (violacein) under QS-control. The different parts of *E. hirta* extracts were then subjected to preliminary phytochemical screening using standard procedures and finally analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Preliminary screening on fresh parts of this herb revealed that leaves exhibited the highest anti-QS activities towards *C. violaceum*. The results also exhibited the wide variation in the anti-QS activities on whole plants, flowers, stems, leaves and roots of *E. hirta* from acetone extraction. The highest anti-QS activities were recorded by leaves and flowers extracts as the lowest of minimum QS inhibition concentration values (1.8906 mg/ml) were indicated by both extracts respectively. Phytochemical screening of *E. hirta* extracts revealed the presence of carbohydrates, lipids, protein, flavonoids, alkaloids, saponins, resins, steroids, acidic compounds, tannins, glycosides, phenols and terpenoids. The quantitave phytochemical assays *via* GC-MS indicated that this herb rich with fatty acids, terpenoids and phenolic compounds.

Keywords: *Euphorbia hirta*, *Chromabacterium violaceum*, anti-quorum sensing, phytochemical assays.

1.0 INTRODUCTION

Euphorbia hirta is widely used as a traditional medicine herb in all the tropical countries (Loh *et al.*, 2009). This herb is categorized as anthropogenic herb which is commonly seen occupying open waste spaces, roadsides, pathways, and as a weed of cultivation (Adedapo *et al.*, 2005). According to Upadhyay *et al.* (2010), different parts of *E. hirta* are used for curing various ailments. The aerial parts of the plant are harvested when in flower during the summer and dried for later use. In East and West Africa, extracts of the decoction of the flowering plants are used in treatment of asthma and respiratory tract infections (Johnson *et al.*, 1999). The stem is used as a treatment for asthma, bronchitis and various other lung complaints. The whole plant is decocted and used in the treatment of athlete's foot, dysentery, enteritis, and skin conditions (Upadhyay *et al.*, 2010).

Recently, this herb is reported to show numerous pharmacological properties such as analgesic, anti-pyretic, anxiolytic, sedative, anti-inflammatory activities, inhibitory action on platelet aggregation, antimutagenicity (Loh *et al.*, 2009) and antioxidant (Basma *et al.*, 2011). Besides, *E. hirta* showed the antibacterial activities from the whole plant and leaves towards certain bacteria. Phytochemical screening revealed that the whole plant consist of tannins, saponins, phenolics, flavonoids, cardiac glycosides, anthraquinone and alkaloids, while the leaves have terpenes, tannins, alkaloids and flavonoids (Ogueke, *et al.*, 2007; El-Mahmood, 2009; Upadhyay *et al.*, 2010). Thus, these findings suggested that some components of *E. hirta* extracts are active in the treatment of various diseases and ailments.

Many pharmacognostical and pharmacological investigations are carried out to identify new drugs or to find new lead structures for the development of novel therapeutic agents in the antibiotic treatments (Newman *et al.*, 2003). A promising approch to find new lead structure is to target bacterial cell-to-cell communication, commonly known as anti-quorum sensing, as the basis of antipathogenic drug treatment (Chong *et al.*, 2010).

Quorum sensing (QS) is a widespread prokaryotic intercellular communication system based on the signal molecules (autoinducers) relative to cell density (Williams *et al.,* 2000). Compounds that interfere with the QS system to attenuate bacterial pathogenicity are termed as anti-QS compounds (Abraham *et al.,* 2011). Inhibition of QS is therefore being considered as a new target for antimicrobial chemotherapy with the current quest on discovering non-toxic QS inhibitors from natural sources (Vattern *et al.,* 2007).

In this context, *E. hirta* is the potential herb to identify new drugs or to find new lead structures for the development of new therapeutic agents in the antibiotic treatments. However the studies on the anti-quorum sensing as well as phytochemical constituents on different parts and whole plant of *E. hirta* are still limited. Therefore, this study was undertaken to assess the potential of *E. hirta* as new therapeutic agents in the antibiotic treatments.

2.0 MATERIALS & METHODS

2.1 Test bacteria

Chromabacterium violaceum (ATCC 12472) was used as a biomonitor strain in the anti-quorum sensing assays. The strain was obtained from ATCC: The Global Bioresource Center, Manassas, Virginia (VA), USA. The bacterium was shipped in the freeze dried form (pellet). Then the pellet was incubated in Nutrient broth (NB; Merck, Germany) at 30°C for 24 h. The bacterium then were streaked on Luria Bertani agar (LB agar; Sigma-Aldrich, USA) and incubated for overnight at 30°C. The isolated colony from each strain of bacteria was further inoculated in Luria Bertani broth (LB broth; Sigma-Aldrich, USA) at 30°C for overnight with shaking.

2.2 Euphorbia hirta extracts

The test sample preparation procedures were done according to Ehsan *et al.* (2009) with modifications. Fresh *E. hirta* were collected and washed thoroughly two to three times with running tap water and then with sterile water followed with the separation of the plants into whole plants and parts needed (flowers, stems, leaves and roots), shade-dried for two weeks, powdered and finally used for extraction. 300 g of powder of plants were soaked with 3000 ml of acetone (extract/solvent ratio = 1:10 w/v) for seven to eight days at room temperature with frequent agitation. Following filtration of the suspension through a Buckner funnel and Whatman filter paper #1, the crude acetone extracts were evaporated in rotary evaporator at 40°C with 65 rpm. The crude extract obtained was then prepared for stock solution at a concentration of 1000 mg/ml by diluting a 1000 mg of crude extract in 1 ml of 99.5% Dimethylsulfoxide (DMSO). The stock solution was then preserved at 4°C in airtight bottle until further used. This stock solution was further diluted to a series of double dilutions with Mueller-Hinton broth (MHB) to produce several range of concentrations needed.

2.3 Preliminary screening of anti-QS from fresh parts of E. hirta

Preliminary screening of anti-QS from fresh parts of *E. hirta* (flowers, stems, leaves and roots) were done based on Adonizio *et al.,* (2006) with modifications. The fresh samples of *E. hirta* in this assay were prepared in three conditions, including whole washed parts, unwashed macerated parts and washed macerated parts. First comparison was done to determine which parts of *E. hirta* (flowers, stems, leaves and roots) presence the anti-QS activity. The second comparison was carried on for identifying the anti-QS activity between whole and macerated samples. The studies on the third comparison were implemented against the washed macerated and unwashed macerated samples in order to recognize the effect of surface microbes and epiphylls towards the anti-QS activities. In this study, 5 ml of molten Soft Top Agar (STA) (1.3 g agar, 2.0 g tryptone, 1.0 g sodium chloride, 200 mL deionised water) were seeded with 100 µL of an overnight LB culture of *C. violaceum*. This was gently mixed and poured immediately over the surface of a solidified Luria Bertani agar (LBA; Sigma-Aldrich, USA) plate as an overlay. The whole washed parts (70% ethanol-washed), unwashed macerated and washed macerated parts were directly placed onto the inoculated LBA plates. These agar plates were then incubated at 30°C for overnight. The ability of *E. hirta* parts in inhibiting microbial growth was detected by observing the ring of turbid or creamy ring of viable cells around the samples (known as “halo”). Loss of purple pigment as *C. violaceum* is indicative of QS inhibition.

2.4 Bioassays for anti-QS of E. hirta extracts

Anti-QS from *E. hirta* extracts (whole plants, flowers, stems, leaves and roots) were determined *via* agar well diffusion method according to Yeo and Tham (2011) with modifications. The concentrations of extracts tested were begun with 1000 mg/ml for screening and then with several concentrations of extracts ranging from 500 to 1.8906 mg/ml (to determine minimum QS inhibition values). In this study, five millimeters of molten STA were seeded with 100 µL of an overnight LB culture of *C. violaceum*. This was gently mixed and poured immediately over the surface of a solidified LBA plate as an overlay. Wells of 5 mm diameter and 5 mm depth were made in the solidified agar using a sterile borer. About 10 µl of test samples at different concentrations were dispensed into the wells and allowed to stand about 15 minutes for pre-diffusion of samples. As control, 10 µl of chloramphenicol at a concentration of 5 mg/ml (positive control) and 99.5% of DMSO (negative control) were also loaded into respective wells for each agar plates. The plates were then incubated at 37ºC for 24 hours. The sensitivity of the test bacteria to the extracts were determined by measuring the diameters of the zone of QS inhibition surrounding the wells in millimeter (mm). The values of minimum QS inhibition concentration from each part of *E. hirta* extracts were recorded by monitoring the first concentration tested values that showed no zone of QS inhibition. All the tests were performed in duplicate.

2.5 Phytochemical screening via standard procedures

Phytochemical screening was carried out according to the standard procedures described by Manjamalai *et al.* (2010) and Tiwari *et al.* (2011) in order to identify the constituents present in acetone extract of *E. hirta* parts and whole plants. Roughly, all the extracts was tested with the 16 types of phytochemical screening tests namely carbohydrates, proteins, lipids, flavonoids, alkaloids, saponins, pholabatannins, resins, sterols, steroids, acidic compounds, tannins, glycosides, anthraquinones, phenols and terpenoids analysis. The present of those phytochemical was identified through the observation of appearance or color changes of certain solutions after mixed with *E. hirta* extracts (whole plants, flowers, stems, leaves and roots).

2.6 Phytochemical analysis via GC-MS

GC-MS technique was used in this study to identify the phytocomponents present in the extract. Five *E. hirta* extracts (whole plants, flowers, stems, leaves and roots) were sent to Chromlab (M) Sdn. Bhd., Selangor, Malaysia for analysis. GC-MS analysis of those extracts was performed using a Varian CP 2800 & 1200 L Quadrupole MS/MS equipped with Factor Four: Capillary column (VF - 5 ms, 30 m, 0.25 mm, 0.25 µm) and pure hydrogen was used as the carrier gas at a constant flow rate of 1ml/min. An injection volume of 2µl was employed with split ratio of 50:1 and the injector temperature was 280°C. The oven temperature was programmed from 50°C (isothermal for 1 min), with an increase of 10°C /min to 180°C, then 20°C/ min ending with a 20 min isothermal at 300°C. Total GC running time was 40 min.

2.7 Statistical analysis

There was no statistical test on preliminary screening of anti-QS from fresh parts of *E. hirta* as the “halo” formation were identified as absent (-), present (+) or highly present (++). The data for bioassay of anti-QS were analyzed by simple arithmetic means and standard deviations (n = 2) of the extracts. No other statistical test was applied for the phytochemical studies to show significance since the extracts were either positive or negative. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name and molecular weight component of the test material was ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

3.0 RESULTS

According to the preliminary screening of anti-QS data, fresh leaves of *E. hirta* exhibited the highest anti-QS activities as the ‘halo’ formation present in washed whole leaves. In addition, the ‘halo’ formation highly present in unwashed and washed leaves. Fresh flowers of *E. hirta* indicated the second highest in the anti-QS activity. This part showed no ‘halo’ formation on washed whole flowers but exhibited highly of halo’ formation in unwashed and washed flowers. Fresh stems and roots indicated similar anti-QS activities which negative results

on washed whole samples but positive on unwashed and washed macerated samples subsequently (**Table 1**).

Based on the results from bioassays of anti-QS, leaves extract showed the highest anti-QS activities as the screening results indicated the biggest zone of QS inhibition (22.50 ± 0.71 mm). Whilst, roots extracts exhibited the lowest QS inhibition as the result was 18.50 ± 0.71 mm. Generally, *E. hirta* extracts indicated certain values of the minimum QS inhibition concentration. The highest anti-QS activities were recorded by leaves and flowers extracts as the lowest of minimum QS inhibition concentration values (1.8906 mg/ml) were indicated by both extracts respectively. Meanwhile, the lowest anti-QS activities were indicated by stems and roots extracts as the highest minimum QS inhibition concentration values (7.5625 mg/ml) was recorded among the extracts (**Table 2)**.

Mostly, the results of phytochemical screening were positive to all *E. hirta* extracts (whole plants, flowers, stems, leaves and roots). However, all the crude extracts of *E. hirta* gave negative results on pholabatannins test. Whilst, the negative result on sterols can be shown on flowers, stems and leaves crude extracts. Besides, the crude extracts of stems and roots gave negative results on anthraquinone (**Table 3**).

Generally, the compounds identified in whole plants extract were 109 compounds. Total compounds detected in leaves and flowers were 105 and 95 compounds respectively. Whilst, roots and stems extracts were and 79 and 33 compounds recorded subsequently. Leaves extracts had 11 major compounds detected. Whilst, stems extracts had eight major compounds. Whole plants and flowers extracts had seven major compounds respectively. Meanwhile, roots extracts had only five major compounds identified (**Table 4)**.

4.0 DISCUSSION

Preliminary screening was implemented to determine whether the anti-QS compounds were actually from the plants themselves or from the epiphyll microorganisms. Thus, the ethanol washed plants materials were compared with unwashed, by placing whole and mashed plants directly onto a prepared *C. violaceum*. Preliminary screening of *E. hirta* from fresh parts indicated that flowers, stems, leaves and roots had the potential as anti-QS agents. Among *E. hirta* parts tested, fresh leaves of *E. hirta* exhibited the highest anti-QS activities as the 'halo' formation present in all condition tested (whole washed parts, unwashed macerated parts and washed macerated parts). The presence of anti-QS activity in washed whole leaves indicated that those compounds also secreted on the leaves surfaces. A comparison between whole and mashed plant materials revealed anti-QS activity major showed in crush plant material. Basically, there was no much difference in anti-QS activity was observed in ethanol-washed and unwashed plants materials. Therefore, there was no effect of surface microbes and epiphylls towards the anti-QS activities from fresh parts of *E. hirta*.

The screening of QS inhibition from *E. hirta* extracts at 1000 mg/ml indicated that the leaves extract showed the highest anti-QS activities among the other parts. However, the zone of QS inhibition from all extracts was not really differed. Since the *E. hirta* extracts were effective at inhibiting QS using *C. violaceum* as biomonitor strain, it might be clarified that the responsible compounds in this herb have multiple or broad-spectrum effects. The determination of minimum QS inhibition concentration towards *E. hirta* extracts by using *C. violaceum* was the interesting idea. Minimum QS inhibition values of certain natural compounds are very important in determination of the dosage and frequency administration of those anti-QS agent. Basically, all *E. hirta* extracts indicated certain values of the minimum QS inhibition concentration. The highest anti-QS activities were recorded by leaves and flowers extracts as the lowest of minimum QS inhibition concentration values were indicated by both extracts. Previous study indicated that, the extracts showed positive result on tannins and phenolic compounds. Adonizio (2008) reported that those secondary metabolites act as natural anti-QS agents. Thus, the abundant of those compounds might be the reason on the highest anti-QS activities.

The treatment of *E. hirta* against *C. violaceum* indicated the effectiveness of anti-QS activities. The present of abundant phytochemicals in this herb with well known antibiotic properties could also potentially posses anti-pathogenic too. Packiavathy *et al.*, (2011) reported, such the anti-pathogenic compounds, in contrast to antibacterial compounds, neither kill bacteria nor stop their growth and are assumed to not result in the development of resistant strains. Instead, these compounds attenuate the expression of the genes responsible for

pathogenesis by interfering with bacterial communication system like the quorum sensing activities. Previous reports indicated the limited study on the specific phytochemicals that responsible as the natural sources of the anti-QS agents. According to Adonizio (2008), many plants or herb exhibited the anti-QS activities like *Pisum sativum* (pea), *Allium sativum* (garlic) and *Daucus carota* (carrot). Some fractionation of those crude extracts was attempted; however, no active compounds were elucidated. Generally, most phytochemicals investigated from herbs which act as anti-QS agents like vannilin, furanones, and ellagitannins belong to the phenolic compounds (Adonizio, 2008; Abraham *et al.,* 2011).

Based on this study, it showed that the phenolic compounds present in this herb like; phenol, 1,2,3-benzenetriol, 3-Methylpyridazine and 2-Furancarbohaldehyde, 5-methyl; basically in the moderate values. The possibilities of synergistic or additive effects among those compounds might contribute to the highest anti-QS activities in this herb as successfully reported throughout this study. Besides, the other phytochemicals such as fatty acids and terpenoids could be the potential as natural sources of anti-QS agents. Thus, the presence of those phytochemicals especially phenolic compounds indicated the interaction between anti-QS and phytochemicals properties of this herb.

5.0 CONCLUSION

The purple pigmentation and its inhibition in *C. violaceum* provided a readily and easily observable phenotype that simplified and facilitated screening for QS inhibition. However, it admittedly only provides qualitative results and does not give much insight as to the active chemical compounds. The biomonitor strain (*C. violaceum*) was chosen here for ease of screening but it does have limitations as a model organism and further work will need specific study of activity against concrete pathological microorganisms that report on clearly defined QS-dependent virulence traits.

REFERENCES

Abraham, S.V.P.I., et al. (2011). Antiquorum sensing and antibiofilm potential of *Capparis spinosa*. *Archives of Medical Research,* 42, 658-668.

Adegoke, A.A., et al. (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products,* 3, 6-12.

Adonizio, A.L., et al. (2006). Anti-quorum sensing activity of medicinal plants in Southern Florida. *Journal of Ethnopharmacology,* 105, 427-435.

Adonizio, A.L. (2008). Anti-quorum sensing agents from South Florida medicinal Plant and their attenuation of *Psedomonas aeruginosa* pathogenicity. *Florida International University Electronic Thesis and Dissertations,* 13, 14-21.

Basma, A.A., et al. (2011). Antioxidant activity and phytochemical screening of methanol extracts of *Euphorbia hirta* (L) *Asian Pacific Journal of Tropical Medicine,* 386-390.

Chong, Y.M., et al. (2010). Malabaricone C from *Mysrista cinnamomea* exhibit anti-quorum sensing activity. *Journal of Natural Products,*74, 2261-2264.

Ehsan, B.R., Vital, A. & Bipinraj, N.K. (2009). Antimicrobial activity of the ethanolic extract of *Bryonopsis laciniosa* leaf, stem, fruit and seed. *African Journal of Biotechnology,* 8, 3565-3567.

El-Mahmood, M.A. (2009). Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. *Journal of Medicinal Plants Research,* 3, 498-505.

Johnson, P.B., et al. (1999). *Euphorbia hirta* leaf extracts increase urine output and electrolytes in rats. *Journal of Ethnopharmacology,* 65, 63-99.

Loh, D.S.Y., Er, H.M. & Chen, Y.S. (2009). Mutagenic and antimutagenic activities of aqueous and methanol extracts of *Euphorbia hirta*. *Journal of Ethnopharmacology,* 126, 406-41.

Manjamalai A., et al. (2010). Analysis of phytochemical constituents and antimicrobial activity of some medicinal plants in Tamilnadu, India. *Global Journal of Biotechnology & Biochemistry,* 5, 120-128.

Newman, D.J., Cragg, G.M. & Snader, K.M. (2003). Natural products as sources of new drugs over the period

1981-2002. *Journal Natural Products*, 66, 1022-1037.

Ogueke, C.C., et al. (2007). Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta*. *Journal of American Science*, 3, 11-16.

Packiavathy, I.A.S.V. et al. (2011). Antibiofilm and quorum sensing inhibitory potential of *Cuminum cyminum* and its secondary metabolite methy eugenol against gram negative bacterial pathogens. *Food Research International*, 45, 85-92.

Tiwari, P., et al. (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia*, 1, 98-106.

Upadhyay, B., Singh, K.P. & Kumar, A. (2010). Pharmacognostical and antibacterial studies of different extracts of *Euphorbia hirta* (L). *Journal of Phytology*, 2, 55-60.

Vattern, D.A., Mihalik, K., Crixell, S.H. & McLean, R.J.C. (2007). Dietary phytochemicals as quorum sensing inhibitors. *Fitoterapia*, 78, 302-310.

Williams, P., Camara, M., & Hardman, A. (2000). Quorum-sensing and the population-dependent control virulence. *Philosophical Transactions of the Royal Society*, 355, 667-680.

Yeo, S.S. & Tham, F. (2012). Anti-quorum sensing and antimicrobial activities of some traditional Chinese medicinal plants commonly used in South-East Asia Malaysian. *Journal of Microbiology*, 8, 11-20.

Table 1**:** Preliminary screening of anti-QS for *E. hirta* parts

Euphorbia hirta parts	Anti quorum sensing effects (a "halo"; clear zone of QS inhibition)		
	Washed whole samples	Unwashed macerated samples	Washed macerated samples
Flowers	-[a]	++[c]	++
Stems	-	+	+
Leaves	+[b]	++	++
Roots	-	+	+

[a]"Halo" formation absent; [b]"Halo" formation present; [c]"Halo" formation highly present

Table 2: Bioassays for anti-QS of *E. hirta* extracts *via* agar well diffusion method

Concentration of *E. hirta* extracts (mg/ml)	Zone of QS inhibition (mm)				
	Whole plant	Flowers	Stems	Leaves	Roots
1000.00	22.00 ± 0.00	20.50 ± 0.71	19.00 ± 0.00	22.50 ± 0.71	18.50 ± 0.71
500.00	20.50 ± 0.71	18.50 ± 0.71	17.50 ± 0.71	20.50 ± 0.71	17.00 ± 0.00
250.00	18.00 ± 0.00	15.50 ± 0.71	14.00 ± 0.00	18.50 ± 0.71	12.50 ± 0.71
125.00	16.50 ± 0.71	14.50 ± 0.71	12.50 ± 0.71	17.00 ± 0.00	11.00 ± 0.00
62.50	15.00 ± 0.00	11.00 ± 0.00	11.50 ± 0.71	15.50 ± 0.71	8.50 ± 0.71
31.25	12.50 ± 0.71	9.50 ± 0.71	7.50 ± 0.71	13.00 ± 0.00	7.00 ± 0.00
15.125	9.00 ± 1.41	7.50 ± 0.71	6.50 ± 0.71	9.00 ± 1.41	6.00 ± 0.00
7.5625	8.00 ± 1.41	7.00 ± 0.00	-	7.50 ± 2.41	-
3.7813	-	6.00 ± 0.00	-	6.50 ± 0.71	-
1.8906	-	-	-	-	-
C5[a]	33.00 ± 0.00	33.00 ± 0.00	33.00 ± 0.00	33.00 ± 0.00	33.00 ± 0.00

[a]C5 = Chloramphenicol (5 mg/ml); [b]- = No zone of QS inhibition

Table 3 : Phytochemical screening of *E. hirta* extracts

Phytochemical	Whole plants	Flowers	Stems	Leaves	Roots
Carbohydrates	+	+	+	+	+
Protein	+	+	+	+	+
Lipid	+	+	+	+	+
Flavonoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	+
Pholabatannins	-	-	-	-	-
Resins	+	+	+	+	+
Sterols	+	-	-	-	+
Steroids	+	+	+	+	+
Acidic compounds	+	+	+	+	+
Tannins	+	+	+	+	+
Glycosides	+	+	+	+	+
Anthraquinone	+	+	-	+	-
Phenols	+	+	+	+	+
Terpenoids	+	+	+	+	+

[+] Phytochemical exist; [-] Phytochemical not exist

Table 4: GC-MS data from *E. hirta* extracts

E. hirta parts	Compounds Identified	RT (min) [a]	Major compounds [b]	Molecular Formula	Peak Area (%)
Whole plants	109	21.619	á-Sitosterol	$C_{29}H_{50}O$	2.8900
		22.272	Lupeol	$C_{30}H_{50}O$	2.8813
		21.638	à-Amyrin	$C_{30}H_{50}O$	2.1750
		14.080	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	1.8190
		15.379	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1.2500
		21.575	9,19-Cyclolanost-24-en-3-ol, (3á)-	$C_{30}H_{50}O$	1.1360
		16.457	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$	1.0800
Flowers	95	15.743	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	11.8127
		16.770	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$	6.7283
		22.272	Lupeol	$C_{30}H_{50}O$	3.6690
		21.619	á-Sitosterol	$C_{29}H_{50}O$	3.2280
		16.824	Octadecanoic acid	$C_{18}H_{36}O_2$	2.9914
		22.186	9,19-Cyclolanost-24-en-3-ol, acetate, (3á)	$C_{30}H_{50}O$	2.2400
		16.776	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	$C_{22}H_{38}O_2$	1.9700
Stems	33	15.649	Undecanoic acid	$C_{11}H_{22}O_2$	4.1290
		15.670	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	3.4540
		16.651	10-Undecyn-1-ol	$C_{11}H_{20}O$	3.2080
		16.728	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$	3.0030
		15.748	Tetradecanoic acid	$C_{14}H_{22}O_2$	2.1660
		16.710	cis,cis,cis-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	1.6300
		16.633	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	1.2840
		22.273	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3á,4à,5à)	$C_{30}H_{50}O$	1.2700
Leaves	105	16.819	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{18}H_{32}O_2$	4.6758
		15.813	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	2.8122
		16.859	Octadecanoic acid	$C_{18}H_{36}O_2$	2.6869
		14.573	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	2.2490
		16.388	Phytol	$C_{20}H_{40}O$	1.8081
		22.264	Lupeol	$C_{30}H_{50}O$	1.6550
		4.015	Phenol	C_6H_6O	1.6510
		16.623	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$	1.5970
		21.625	á-Sitosterol	$C_{29}H_{50}O$	1.4240
		22.179	9,19-Cyclolanost-24-en-3-ol, acetate, (3á)	$C_{30}H_{50}O$	1.1440
		16.823	11,14,17-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	1.1300
Roots	79	16.691	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$	3.6100
		16.756	Octadecanoic acid	$C_{18}H_{36}O_2$	2.0630
		15.697	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1.8240
		22.317	Lupeol	$C_{30}H_{50}O$	1.4200
		15.698	n-Decanoic acid	$C_{10}H_{22}O_2$	1.3000

[a] Retention time (min)

[b] The major compounds having 90% comparison with the compounds in the NIST library