LC/MS characterization of flavonoids from leaves of *Clerodendrum inerme* L. cultivated in Iraq.

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ABSTRACT

Objective: The goal of this study was to determine the qualitative and quantitative characteristics of flavonoid compounds extracted from *Clerodendrum inerme* leaves cultivated in Iraq. **Methods:** The plant's leaves were obtained from AL-Musayyab area (south of Baghdad) where the species is well-known. 80% ethanol was used to extract powdered plant leaves. The extract was filtered before being dried using a rotary evaporator. **Results:** The peaks identified according to the order of their retention time were represented by the LC chromatogram obtained for the leaves extract of *C. inerme*. The flavonoids luteoline, hispudulin, vitexin, eupatilin, and baicalien have the greatest peaks among the main compounds. **Conclusion:** When compared to semi-arid places like south of Baghdad, the proportion of flavonoid compounds in *Clerodendrum inerme* leaves in our soil was determined to be satisfactory and just slightly lower than humid regions.

Keywords: LC/MS, Leaves, Flavonoids, *Clerodendrum inerme.*

Introduction:

Historically, natural products have been used to cure a variety of ailments and disorders since antiquity. Natural product chemistry techniques enable the discovery of a large number of bioactive secondary metabolites from both terrestrial and marine sources. As a result, natural products are viewed as an important source of drug development for a variety of diseases [(1)]. Medicinal plants are now recommended in almost all pharmacopoeias for self-medication or by a doctor or pharmacist. They may be used on their own or in combination with other treatments [(2)]. *Clerodendrum inerme* belongs to the Lamiaceae family and is regarded a significant food and medicinal plant [(3)]. It's grown wildly in middle region of Iraq and called Yasmine Kathib which is a plant that is utilized in traditional medicine and as food. Yasamen Zefar and Shajar khat are two alternative local names for this plant [(4&5)]. The plant grows in tropical and subtropical climates across the globe. Flavonoids, glycosides, anthraquinones, steroids, monoterpenes, diterpenes, triterpenes, saponins, and volatile oils were found in *Clerodendrum inerme* [(6)]. Fruits, vegetables, grains, bark, roots, stems, flowers, and tea all

contain flavonoids, which are a collection of natural compounds with varying phenolic structures. Because these natural chemicals are well-known for their health benefits, researchers are working to extract the flavonoids. Flavonoid compounds are plant extracts that may be found in many areas of the plant in nature [(7)]. Antimicrobial, anti-inflammatory, anti-malarial, anti-diabetic, anti-cancer, analgesic, and anti-oxidant properties have been discovered in the many species of the genus [(8)].

Figure (1) *Clerodendrum inerme* L leaves [(5)].

Materials and methods:

Plant material:

The leaves of *C. inerme* were taken from shrubs in Al-Musayyab region's public gardens. The plant was authenticated in the College of Science's herbarium at the University of Baghdad. In September, the plant material was gathered, dried at room temperature in the shade, then ground into a powder and weighed.

Extraction of the plant material:

100 gram of powdered plant leaves extracted with 800 ml of ethanol 80% after hexane extraction using soxhlet apparatus for 16 hours. The extract was filtered and then evaporated to dryness by rotary evaporator.

Liquid Chromatography/ Mass Spectrometry (LC/MS):

At the College of Pharmacy/Zarqa University in Jordan, liquid mass detection was done using a Bruker Daltonik Impact II ESI-Q-TOF System coupled with a Bruker Daltonik Elute UPLC system (Bremen, Germany) for screening several chemicals of interest.

Ion Source Apollo II ion Funnel electrospray source was used to run the device. The capillary voltage was 2500 V, the nebulizer gas pressure was 2.0 bar, the dry gas (nitrogen) flow rate was 8 L/min, and the dry temperature was 200 degrees Celsius. The mass resolution was 50000 FSR (Full Sensitivity Resolution), while the TOF repetition rate was up to 20 kHz. The mass accuracy was less than 1 ppm.

Standards for high-resolution m/z identification Bruker TOF MS and stock solutions were made by dissolving the necessary quantity of material in Dimethyl sulfoxide-DMSO (analytical grade), dilution with Acetonitrile, and use for MS and retention time identification. Acetonitrile, methanol, water, and formic acid were all employed as LC/MS grade reagents.

Results and discussion:

1- Preliminary Chemical test (Alkaline Reagent Test) for flavanoids.

To 1 mL of ethanolic extract of the plant, 2-3 mL of alcoholic KOH were added. If flavonoids compounds are present, a yellow color is noticed, after which acid is added and the color is masked. The presence of flavonoids in the ethanol extract was discovered during early phytochemical screening of the plant extract. As a result, LC-MS was used to confirm this, as well as to establish which flavonoid is the most abundant in Iraqi *Clerodendrum inerme.*

2- LC/MS characterization of active compounds:

To create ethyl acetate extract, ethanolic extract was fractionated with ethyl acetate solvent and water to improve polarity. For screening several compounds of interest, liquid mass detection was conducted using a Bruker Daltonik Impact II ESI-Q-TOF System coupled with a Bruker Daltonik Elute UPLC system (Bremen, Germany). Standards for m/z identification with a high-resolution Bruker TOF MS and stock solutions were made by dissolving the necessary quantity of material in Dimethyl sulfoxide-DMSO (analytical grade), dilution with Acetonitrile, and use for m/z identification.

Figure 2 shows the LC chromatogram produced for *Clerodendrum inerme* leaves, with peaks identified according to the order of their retention time. The retention duration of standards, [M H] data, MS/MS fragmentation peaks, and published data were used to characterize the structure.

The retention time, m/z measures, M measures, [M − H]− peaks, molecular formula for each compound (1-11) are shown in table 1. Also, fragmentation pattern of some of the most significant compounds are shown in figure 4.

The major compounds included as flavonoids are luteoline, hispudulin, vitexin, eupatilin, and baicalien based on the greatest peaks, which is consistent with studies done on the plant that is grown in other countries [(9)]. Also, other compounds as dicarboxylic acid compound succinic acid and flavonoid glycosides as Luteolin 7-O-glucoside are found to be present in considerable percentages.

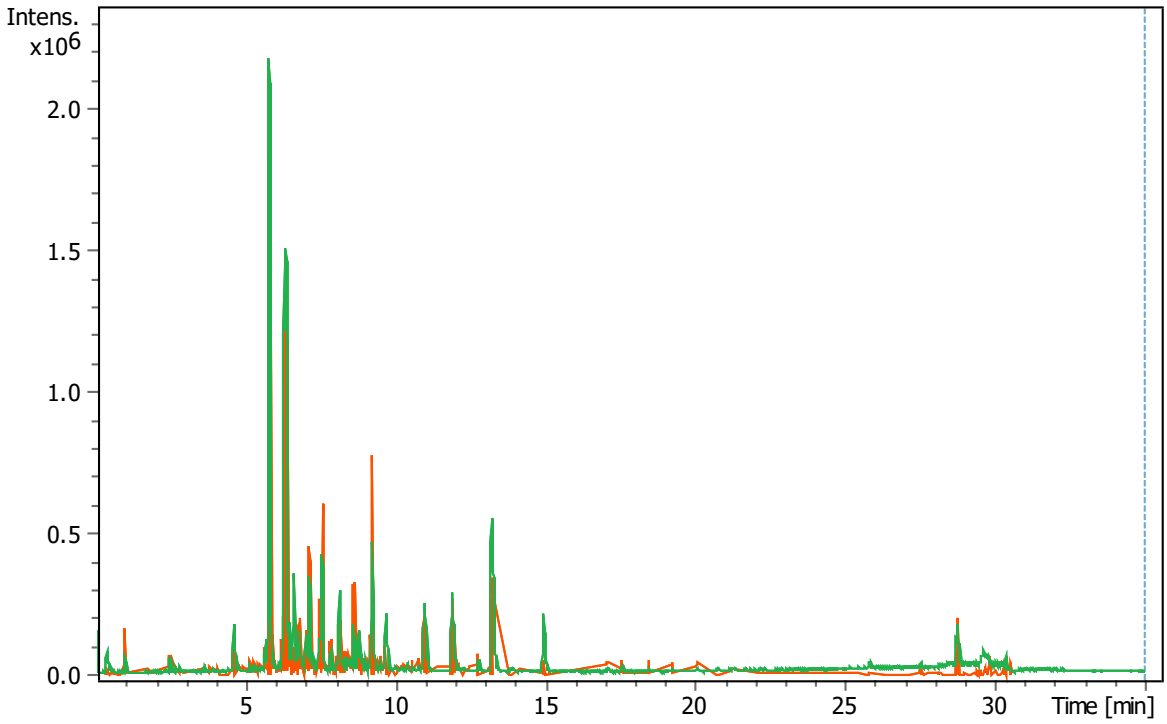


Figure 2: LC-chromatogram obtained for the leaves of *Clerodendrum inerme*

Table (1): the retention time, m/z measures, M measures, [M − H]− peaks, molecular formula for each compound (1-11)

Peak No	Compound	RT(min)	m/z meas.	M meas.	Ions	Molecular formula
1	5,6,4'-Trihydroxy-7,3'-dimethoxyflavone	10.54	329.06648	330.07375	M-H	C17H14O7
2	7-Glu Chrysoeriol	8.35	461.10901	462.11628	[M-H]-	C22H22O11
3	Acteoside	5.94	623.19841	624.20546	[M-H]-, [M-H-H]2-	C29H36O15
4	Baicalein	9.83	269.04556	270.05284	[M-H]-	C15H10O5
5	Eupatilin	12.41	343.08232	344.08959	[M-H]-	C18H16O7
6	Hispudulin	10.34	299.05593	300.06321	[M-H]-	C16H12O6
7	Vitexin	5.66	431.09821	432.10548	[M-H]-	C21H20O10
8	Luteolin	8.54	285.04032	286.04759	[M-H]-	C15H10O6

9	Luteolin 7-O-glucoside (Cynaroside)	5.82	447.0933 1	448.1005 9	[M-H]-	C21H20O11
10	Sorbifolin	11.11	299.0560 6	300.0633 4	[M-H]-	C16H12O6
11	Succinic acid	0.98	117.0195 6	118.0268 3	[M-H]-	C4H6O4

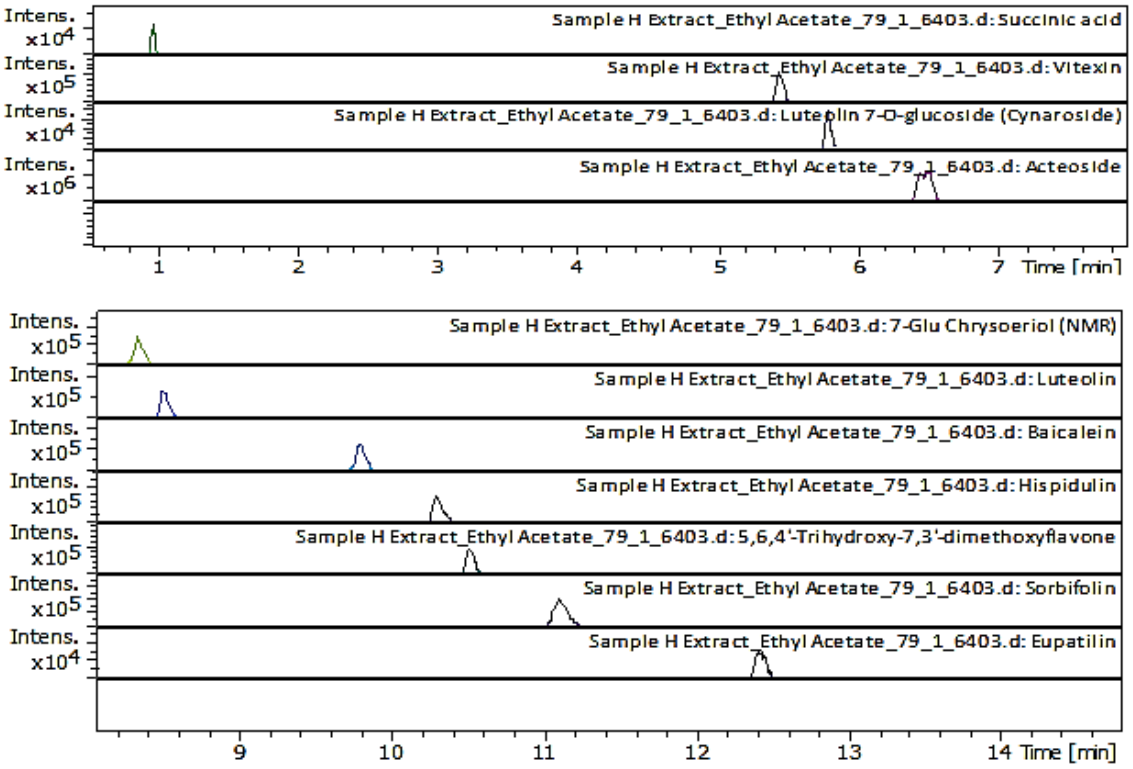
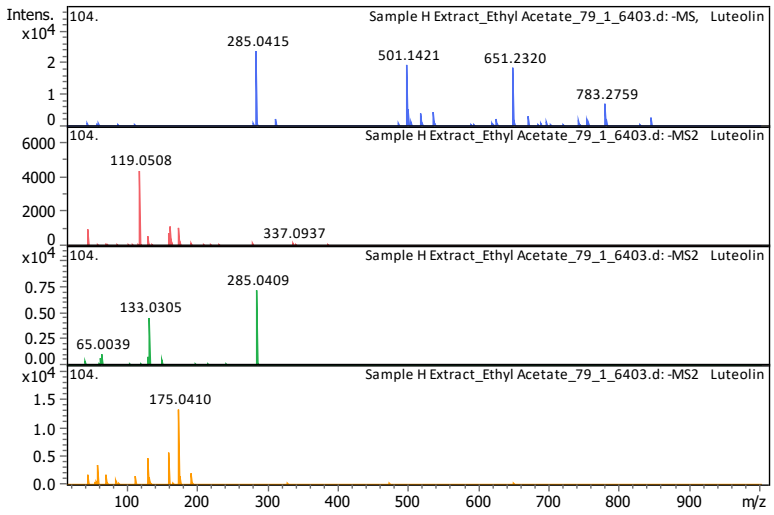
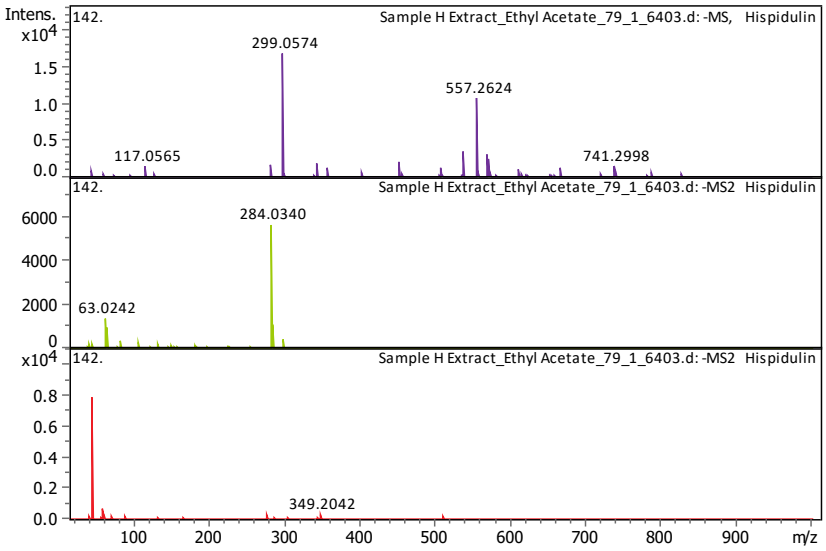


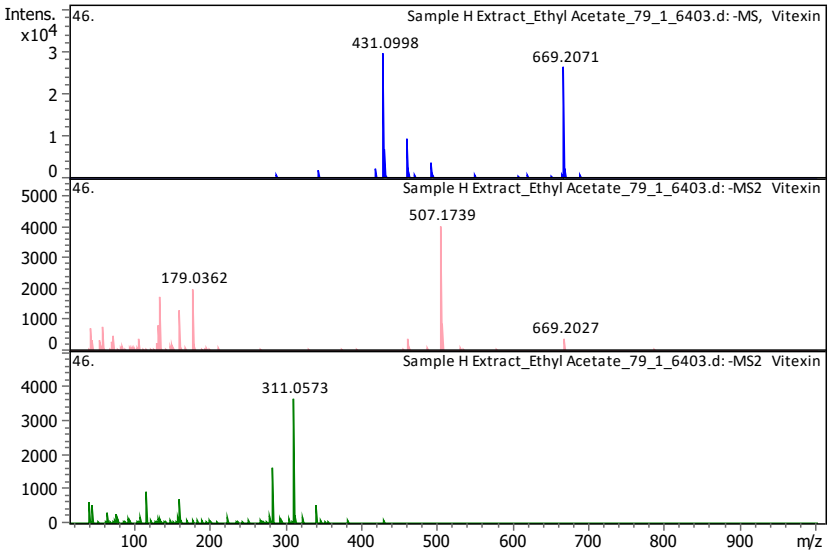
Figure 3: LC-chromatogram of each individual peak of the main compounds obtained for the leaves of *Clerodendrum inerme*



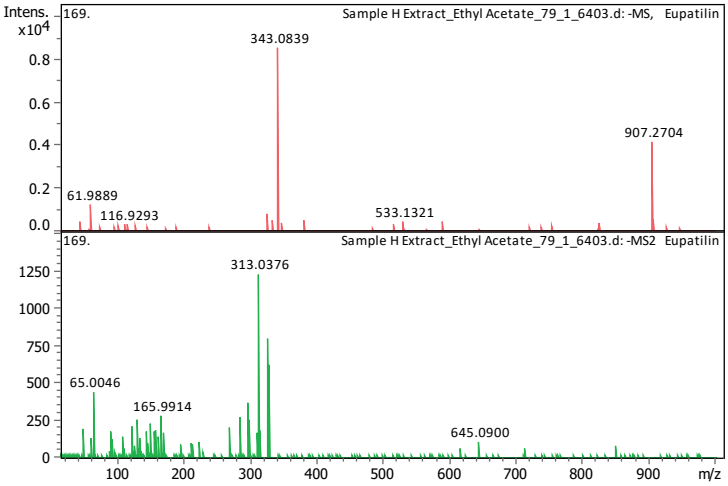
(A)


Intens.
x10^4
142.
Sample H Extract_Ethyl Acetate_79_1_6403.d: -MS, Hispidulin
299.0574
557.2624
117.0565
741.2998
142.
Sample H Extract_Ethyl Acetate_79_1_6403.d: -MS2 Hispidulin
284.0340
63.0242
142.
Sample H Extract_Ethyl Acetate_79_1_6403.d: -MS2 Hispidulin
349.2042
m/z

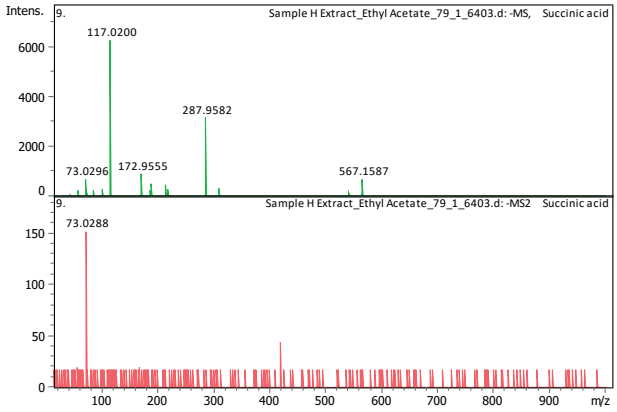
(B)


Intens.
x10^4
46.
Sample H Extract_Ethyl Acetate_79_1_6403.d: -MS, Vitexin
431.0998
669.2071
46.
Sample H Extract_Ethyl Acetate_79_1_6403.d: -MS2 Vitexin
507.1739
179.0362
669.2027
46.
Sample H Extract_Ethyl Acetate_79_1_6403.d: -MS2 Vitexin
311.0573
m/z

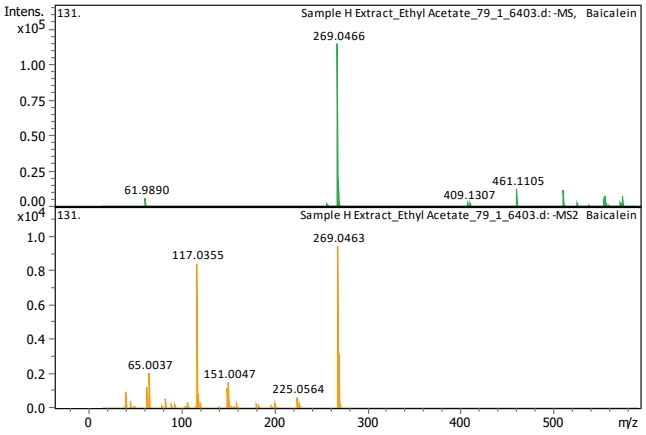
(C)


Intens.
x10^4
169.
Sample H Extract_Ethyl Acetate_79_1_6403.d: -MS, Eupatilin
343.0839
907.2704
61.9889
116.9293
533.1321
169.
Sample H Extract_Ethyl Acetate_79_1_6403.d: -MS2 Eupatilin
313.0376
65.0046
165.9914
645.0900
m/z

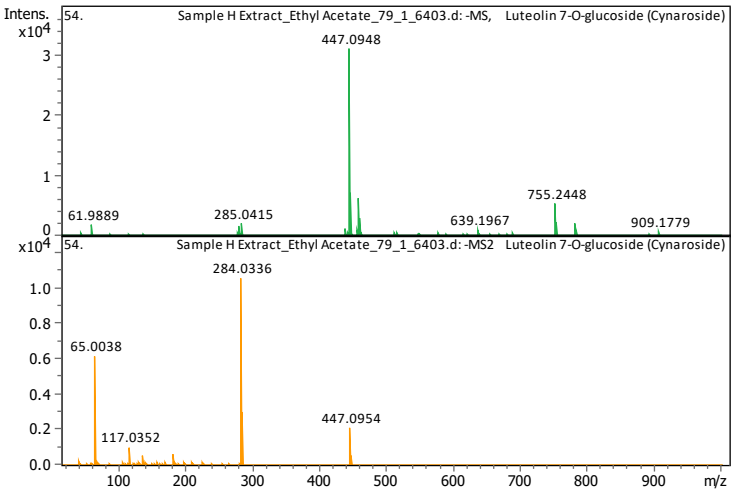
(D)



(E)



(F)



(G)

Figure 4: Fragmentation patterns of some of the most significant compounds: (A) Luteolin, (B) Hispidulin, (C) Vitexin, (D) Eupatilin, (E) Succinic acid, (F) Baicalein, (G) Luteolin 7-O-glucoside obtained from the leaves of *Clerodendrum inerme* L.

Conclusion:

When compared to semi-arid places like south of Baghdad, the proportion of flavonoid compounds in *Clerodendrum inerme* leaves in our soil was determined to be satisfactory and just slightly lower than humid regions.

ACKNOWLEDGMENTS

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References

1. Tawfeeq TA, Jasim GA, Nasser AA. Isolation of umbelliferone from leaves of *Conocarpus erectus* L. cultivated in Iraq. 2020;20(4):82–92.
2. Eldalawy R, Al-ani WM. GC-MS analysis of Iraqi *Silybum marianum* Flowers, Leaves and Seeds Extracts. 2020;20(4):93–112.
3. Uritu CM, Mihai CT, Stanciu GD, Dodi G, Alexa-Stratulat T, Luca A, et al. Medicinal plants of the family Lamiaceae in pain therapy: A review. Pain Res Manag. 2018;2018:1–44.
4. Starr F, Starr K, Loope L. *Clerodendrum inerme.* Museum. 2003;(1999):1997–9.
5. Wight N, Used O, Folk IN. Research article INVESTIGATIONS ON DIFFERENT PARTS OF BULBOPHYLLUM. 2013;2(4):259–69.
6. Ibrahim SRM, Alshali KZ, Fouad MA, Elkhayat ES, Al Haidari RA, Mohamed GA. Chemical constituents and biological investigations of the aerial parts of Egyptian *Clerodendrum inerme*. Bull Fac Pharmacy, Cairo Univ. 2014;52(2):165–70.
7. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci. 2016;5(47):15–1.
8. Kar P, Goyal AK, Das AP, Sen A. Antioxidant_and_pharmaceutical_potential. 2014;(September):7–1.
9. Huang WJ, Lee HJ, Chen HL, Fan PC, Ku YL, Chiou LC. Hispidulin, a constituent of *Clerodendrum inerme* that remitted motor tics, alleviated methamphetamine-induced hyperlocomotion without motor impairment in mice. J Ethnopharmacol [Internet]. 2015;166:18–22. Available from: http://dx.doi.org/10.1016/j.jep.2015.03.001