Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

HPLC-PDA-ESI-MS-MS Analysis of Acids Content of *Lantana camara* L. Flower Extract and Its Anticoagulant Activity

S. N. El Gendy[1], M. I. Ezzat[1], A. M. EL Sayed[1], A. S. Saad[2], A. K. Elmotayam[1*]

[1] *Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr El-Ainy Street, Cairo 11562, Egypt.*

[2] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Port Said University, Port Said, Egypt*

Abstract

Lantana camara L. (Family Verbenaceae) is an ornamental herb found in tropical and sub-tropical countries. It has been widely used in traditional medicine for the treatment of various illnesses. *L. camara* contains a large number of phytochemical compounds. Polyphenol metabolites of *L. camara* L. are the major bioactive constituents. Recently scientifically proven therapeutic activities of *L.camara* include antibacterial, antioxidant, antipyretic, insecticidal, antimicrobial, wound healing, and anti-urolithiatic activities. Limited information is available regarding the phytoconstituents and other health value of *Lantana* flowers. The fragmentation behavior of phenolic acid derivatives and other acids was investigated using HPLC-PDA-ESI-MS in negative mode where even minor components were detected due to the high sensitivity of mass spectrometer detector. The given acids profile led to discovery of many unreported metabolites including hydrogen-bonded dimers of acids and acids bound to sugars. The anti-coagulant activity measured by estimation of prothrombin time (PT) using normal citrated human plasma and heparin as positive control revealed that *Lantana* flowers and roots extracts increased the clot time relative to the control. This activity may be attributed to the high-phenolic content of the flowers as many studies have reported.

Keywords: *Lantana camara*, HPLC-PDA-MS/MS, phenolic acids, anticoagulant.

1. Introduction

Using of plants as remedies for many diseases has formed the base of the modern medicine. According to WHO (2008), approximately 80% of Africa and Asia's population use different botanicals for treatment of various diseases including blood disorders. They warranted a rich source of bioactive metabolites belonging to various classes of natural compounds.

Lantana camara L. is reputable source of polyphenol constituents which attracted several investigators for studying its phytochemical phytochemistry study and revealing their its activities. The flavonoids, phenylethanoids profiling and iridoids of the flower extract was investigated and evidenced several unreported metabolites [1]. The flower extract also proved to be beneficial for human health especially in dissolution of oxalate kidney stones [1]. Meanwhile, such study revealed the existence of no lantadenes which possess hepatotoxic activity [2]. This may promote *Lantana* flowers to be candidate as food supplement for human consumption and provide comprehensive data for quality evaluation. On continuation searching of flower content of *L. camara*, our study aimed to investigate the acid content using LC/MS.

Phenolic acids are a group of non-flavonoid polyphenols that play important role in growth, development, and reproduction. They are widely distributed in plant kingdom as secondary metabolites [3]. All phenolic acid compounds have a carboxyl group linked to a benzene ring. They may be either benzoic acid derivatives or cinnamic acid derivatives [4].

The value of phenolic compounds in human diet was attributed to their antioxidant properties[5]. Many recent researches revealed their biological and therapeutic activities as antibacterial[6], anti-inflammatory[7], antiviral [8] and anticancer activities[9]. Additionally, many studies reported and evaluated the hemostatic and anticoagulant activities of phenolic acids by several bioassays[10][11].

*Corresponding author e-mail: amira.kamal@pharma.cu.edu.eg.; (A. K. Elmotayam).
Receive Date: 24 March 2022, Revise Date: 14 April 2022, Accept Date: 19 April 2022.
DOI: 10.21608/EJCHEM.2022.129350.5715

Phenolic acids could also participate as precursors in developing new hemostatic and anticoagulant drugs[12][13]. prothrombin time (PT) assay is one of the most widely used assays for monitoring the effects of conventional oral anticoagulants [14][15].
Search for safe and effective anticoagulants from plants continues with promising finding. Polyphenols have been shown to be the main constituents which inhibit platelet aggregation and reactive oxygen species (ROS) production. This study was undertaken to identify other components in *Lantana* flowers and report their anticoagulant activity.

2. Experimental

2.1 Plant materials:

The leaves, flowers and roots of *L. camara* were collected in September 2016, from the Experimental Station of Medicinal, Aromatic and Poisonous plants, Department of Pharmacognosy, Faculty of Pharmacy, Cairo University. The plant material was authenticated by Professor Abd-Halim Megaly, Professor of plant taxonomy agriculture museum. A voucher specimen was placed in the Herbarium of Pharmacognosy Department, College of Pharmacy, Cairo University, Egypt. (30-10-2016).

2.2 The plant extracts preparation:

The different parts of *Lantana camara* were air dried under normal environmental conditions and then subjected to size reduction to get coarse powder. The dried powders (700 g of leaves, 300 g of flowers, and 211 g of roots) were separately extracted with ethanol 70% by cold maceration till complete exhaustion. The ethanol extract of each organ was evaporated under reduced pressure at a temperature not exceeding 40 ºC to yield the corresponding extractives, viz., leaves ethanol extract (LE, 75.7 g), flowers ethanol extract (FlE, 128.3g) and roots ethanol extract (RE, 7.9 g).

2.3 HPLC-PDA-MS/MS conditions:

The sample was injected to HPLC hyphenated with mass spectrometer. LC-PDA-MS analysis system consists of HPLC thermofingan (Ther electron corporation, USA) and LCQ-Duo ion trap mass spectrometer with an ESI source (ThermoQuest).
Optimal separation was achieved using Zorbax Eclipse XDB-C18 reversed phase column (150 x 4.6 mm. internal diameter; 3.5 µm.particle size) (Agilent, USA). The mobile phase for gradient elution consists of water and acetonitrile (with 1% formic acid) which was applied from 5% to 30% acetonitrile in 60 min. The flow rate was 1 ml /min throughout the whole run. The samples were automatically injected using autosampler thermoquest. The negative ion mode parameters were as follows: source temperature 200°C, capillary voltage of -10 V, sheath gas flow rate 80 arbitrary units, auxiliary gas flow rate 40 arbitrary units. The spectra were recorded in a full scan mode and mass range of 50-2000 m/z. The peaks and spectra were processed using Thermo Xcalibur software which was used to collect the UV chromatogram using PDA mode. The compounds were tentatively identified by comparing their retention time (Rt) and mass spectra with the literature.

2.4 *In vitro* Anticoagulant Activity:

2.4.1 Collection of blood and separation of plasma:

Blood samples were drawn from healthy volunteers via vein puncture. The blood was collected in containers containing trisodium citrate solution to prevent the natural coagulation process. Immediately, centrifugation was carried out to separate the blood cells from plasma and obtain pure platelet plasma for prothrombin time test.

2.4.2 Tested Extracts:

Ethanolic extract of flowers, roots and leaves were investigated for their anticoagulant activity. Each extract was prepared in four concentrations 1, 0.5, 0.25, 0.125 mg/ml in dimethyl sulfoxide**.**

2.4.3 Experimental design:

Plasma sample was divided into 14 groups:

- Group I: Positive control group 0.2 ml of plasma + 0.1ml of 1U/ml of heparin
- Group II: Negative control group: 0.2 ml plasma + 0.1 ml of DMSO
- Group III: 0.2 ml of plasma + 1 mg/ ml of flowers extract (F_1)
- Group IV: 0.2 ml of plasma + 0.5 mg/ml of flowers extract (F_2)
- Group V: 0.2 ml of plasma + 0.25 mg/ml of flowers extract (F_3)
- Group VI: 0.2 ml of plasma + 0.125 mg/ml of flowers extract (F_4)
- Group VII: 0.2 ml of plasma + 1 mg/ ml of roots extract (R_1)
- Group VIII: 0.2 ml of plasma + 0.5 mg/ml of roots extract (R_2)
- Group IX: 0.2 ml of plasma + 0.25 mg/ml of roots extract (R_3)
- Group X: 0.2 ml of plasma + 0.125 mg/ml of roots extract (R_4)
- Group XI: 0.2 ml of plasma + 1 mg/ ml of leaves extract (L_1)
- Group XII: 0.2 ml of plasma + 0.5 mg/ml of leaves extract (L_2)
- Group XIII: 0.2 ml of plasma + 0.25 mg/ml of leaves extract (L_3)

- Group XIV: 0.2 ml of plasma + 0.125 mg/ml of leaves extract (L_4)

All plant extracts were added separately to the plasma samples in a water bath at 37 °C with gentle shaking. Then thromboplastin reagent was added. The prothrombin time (time of clot formation) was measured using stop watch.

2.4.4 Statistical analysis:

All values were presented as means ± standard error of the means (SEM) with n=7. Statistical analysis was performed using GraphPad Prism version 5 (GraphPad, San Diego, California, USA). Comparison between groups was carried out using one-way analysis of variance (ANOVA), followed by the post-hoc Dunnett's test to analyze the data. Difference was considered significant when $P < 0.05$.

3 Results

3.1 HPLC-PDA-MS/MS

Acid content of *Lantana camara* L. flowers:

Thirty two acids were identified in *L. camara* L. flowers. They were of three main categories namely, phenolic acids, non-phenolic acids and oxygenated fatty acids. Results of HPLC-PDA-MS/MS analysis is recorded in table (1) and figure (1).

Table 1: Identified acids in *L. camara* L. flower extract using HPLC-PDA-ESI-MS/MS in negative mode.

No	Rt (min)	λ_{max} (nm)	$(M-H)^-$	Mass fragmentation	Identified compounds	Ref.
1	1.12	-	133	115	Malic acid	[22]
2	2.31	-	217	173,137,97,93	*P*-hydroxybenzoic acid sulfate	
3	2.67	-	131	113,87,77,45	glutaric acid	[22]
4	2.93	256, 285	145	127,101,83,57	Adipic acid	[22]
5	3.44	294	299	137,93	*P*-hydroxybenzoic acid hexoside	[16]
6	4.24	284	153	109	Protocatchuic acid	[16]
7	5.19	276	207	207,179,161,135	Caffeic acid ethyl ether	
8	6.48	201,256, 291	153	109	Gentisic acid	[17]
9	6.94	276	167	152,149,123,109	Vanillic acid	[16]
10	9.24	252	137	93	P- hydroxy benzoic acid	[19]
11	10.42	252	179	161,135,109,91	Caffeic acid	[19]
12	10.82	251	223	208,205,179	Sinapic acid	[21]
13	12.85	268,318	401	269,161	Caffoeyl benzyl alcohol pentoside	
14	13.72	270	163	119	*P*- coumaric acid	[20]
15	14.32	285	225	207,181,165	Dihydrosinapic acid	[18]
16	16.15	220, 284	173	137,129,111	Shikimic acid	[22]
17	17.84	221, 284	325	179,163,161,119	Caffoeyl rhamnose	
18	19.39	226	359	223,179,135	Caffeic acid dimer (Rosmarinic acid)	[20]
19	22.70	209,222, 297	163	119	O-Coumaric acid	
20	27.58	290	475	341,323,299,203,179,161,151,135	Caffeic acid-3-O-anisoyl-4-O-hexoside.	
21	32.43	322	181	181,153,152,137,109	Protocatechuic acid dimethyl ether	[18]
22	35.78	313	445	401,307,281,163, 137	p -hydroxy benzoic acid rutinoside	
23	37.93	294, 315	475	307,167	Vanillic acid rutinoside	
24	42.97	299, 309	471	325,307,163,145	p-coumaric acid rutinoside	
25	44.83	316	561	441,415,269,163	Coumaric-p-hydroxy benzyl dirhamnoside	
26	46.48	322	591	445,429,325,307	Benzoyl caffeic acid rutinoside	
27	66.97	225, 316	307	289 ,235	Hydroxy - oxo-octadecatrienoic acid	[23]
28	67.27	228, 270, 281, 316	309	291	Dihydroxy-octadecatrienoic acid	[24]
29	70.01	228, 332	311	293	Dihydroxy-octadecadienoic acid	[25]
30	72.81	228, 332	313	295 ,183	Dihydroxy octadecenoic acid	[26]
31	77.26	234	293	275,171	Hydroxy-octadecatrienoic acid	[24]
32	79.88	233	295	277 ,171	Hydroxy-octadecadienoic acid	[24]

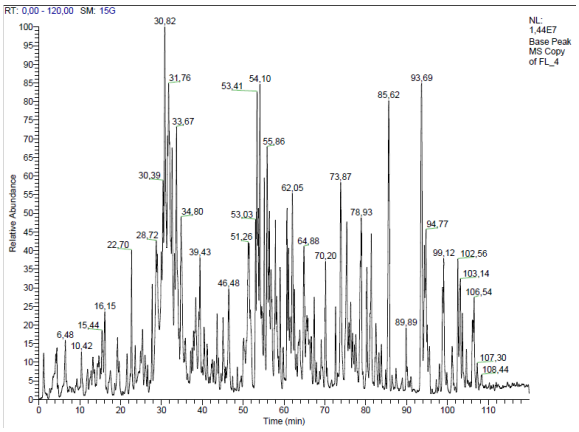

Fig. (1): Representative HPLC-PDA-ESI-MS/MS of *Lantana camara* L. flowers in negative mode.

Phenolic acids:

Peak 6 and peak 8 (*m/z* 153 $C_7H_5O_4^-$) showed loss of CO_2 to give the fragment ion at *m/z* 109. They were identified as protocatechuic acid [3] and its isomer gentisic acid [4]. Protocatechuic acid was detected also in its dimethyl ether form in peak 21 (*m/z* 181 $C_9H_9O_4^-$) [5].

Peak 10 (m/z 137 $C_7H_5O_3^-$) showed a 44 amu (CO_2) loss to give m/z 93. It was identified as *p*-hydroxy benzoic acid [6]. Peak 2 (*m/z* 217 $C_7H_5O_7S^-$), peak 5 (*m/z* 299 $C_{13}H_{15}O_8^-$) and peak 22 (*m/z* 445 $C_{22}H_{21}O_{10}^-$) exhibited loss of 80 amu (M-H-SO_4), 162 amu (M-H-hexose) and 308 amu (M-H-rutinoside) respectively, to give the base peak at *m/z* 137. They were identified as *P*-hydroxybenzoic acid-4-*O*-sulphate, *p*-hydroxy benzoic acid hexoside [3] and *p*-hydroxy benzoic acid rutinoside respectively .

Peak 11 (*m/z* 179 $C_9H_7O_4^-$) showed a base peak at *m/z* 135 (M-H-CO_2^-) and fragment ion at *m/z* 161 (M-H-18^-). It was identified as caffeic acid [19]. Caffeic acid was identified in its dimeric form (Rosmarinic acid) at peak 18 (*m/z* 359 $C_{18}H_{16}O_8^-$) [20]. Peak 7 (*m/z* 207 $C_{11}H_{11}O_4^-$) showed a fragment ion at *m/z* 179 (M-H-28^-) which was attributed to the loss of ethyl group. It was identified as caffeic acid ethyl ether. Peak 17 (*m/z* 325 $C_{18}H_3O_6^-$) showed a base peak at *m/z* 179 (M-H-146^-) which was attributed to the loss of deoxy hexose moiety. It was identified as caffoeyl rhamnose. Peak 13 (*m/z* 401 $C_{20}H1_7O_9^-$) produced a base peak at *m/z* 269 (M-H-132^-) as a result of the loss of a pentose moiety. The subsequent loss of 108 amu (benzyl alcohol) resulted in the fragment ion at *m/z* 161 (M-H-132- 108^-). It was identified as caffoeyl benzyl alcohol pentoside. Peak 20 (*m/z* 475 $C_{23}H_{23}O_{11}^-$) showed a distinctive peak at *m/z* 341 (M-H-134^-) which was attributed to the loss of anisoyl moiety. The further loss of 162 amu (hexose moiety) produced the fragment ion at *m/z* 179 (M-H-134-162^-). It was identified as caffeic acid-3-*O*-anisoyl-4-*O*-hexoside.

Peak 9 (*m/z* 167 $C_8H_7O_4^-$), peak 12 (*m/z* 223 $C_{11}H_{11}O_5^-$) and peak 15 (*m/z* 225 $C_{11}H_{13}O_5^-$) showed a 44 amu (CO_2) loss to give *m/z* 123, *m/z* 179 and *m/z* 181 respectively. They were identified as vanillic acid , sinapic acid and dihydrosinapic acid respectively [3] [8] [5].

Peak 14 (*m/z* 163 $C_9H_7O_3^-$) and Peak 19 (*m/z* 163 $C_9H_7O_3^-$) exhibited a base peak at *m/z* 119 (M-H-44^-) due to the loss of CO_2. They were identified as p-coumaric acid and its isomer o-coumaric acid [7].

Peak 23 (*m/z* 475 $C_{20}H_{27}O_{13}^-$) and peak 24 (*m/z* 471 $C_{21}H_{27}O_{12}^-$) exhibited a loss of 308 amu (rutinoside moiety) to give *m/z* 167 and *m/z* 163 respectively. They were identified as vanillic acid rutinoside and p-coumaric acid rutinoside respectively. Peak 25 (*m/z* 561 $C_{27}H_{29}O_{13}^-$) characterized by two sequential losses of 146 amu (deoxyhexose) to give the product ion at *m/z* 269. The further loss of 106 amu (p-hydroxy benzyl) resulted in the distinctive peak at *m/z* 163 (coumaric acid). It was identified as coumaric p-hydroxy benzyl dirhamnoside. Peak 26 (*m/z* 591 $C_{28}H_{31}O_{14}^-$) showed a base peak at *m/z* 445 (M-H-146^-) which was attributed to the loss of deoxyhexose moiety. The subsequent loss of 162 amu gave the fragment ion at *m/z* 283 (M-H-146-162^-). The further loss of 104 amu (benzoyl moiety) produced an intense ion at *m/z* 179 (caffeic acid). It was identified as benzoyl caffeic acid rutinoside.

Non phenolic acids:

Four non phenolic acids were identified in the ethanol extract of *L. camara*. L. flowers. Peak 1 (*m/z* 133 $C_4H_5O_5^-$) gave a base peak at *m/z* 115 (M-H- $H_2O)^-$. It was identified as malic acid. Peak 3 (*m/z* 131 $C_5H_7O_4^-$) showed two fragments at *m/z* 113(M-H- $H_2O)^-$ and *m/z* 87 (M-H-$CO_2)^-$. It was determined as glutaric acid. Peak 4 (*m/z* 145 $C_6H_9O_4^-$) showed product ions at *m/z* 127 (M-H- $H_2O)^-$ and *m/z* 101(M-H-$CO_2)^-$. It was defined as adipic acid. Peak 16 (*m/z* 173 $C_7H_9O_5^-$) showed base peak at *m/z* 111 (M-H- H_2O - $CO_2)^-$. It was defined as shikimic acid [9].

Oxygenated fatty acids:

Peaks 27 (*m/z* 307 $C_{18}H_{27}O_4^-$) and 28 (*m/z* 309 $C_{18}H_{29}O_4^-$) showed a mass difference of 2 amu which is indicative of dehydrogenation of one of the hydroxy groups and were identified as Hydroxy-oxo-octadecatrienoic acid and Dihydroxy-octadecatrienoic acid respectively [10] [11].

Peaks 29 (*m/z* 311 $C_{18}H_{31}O_4^-$) and 30 (*m/z* 313 $C_{18}H_{33}O_4^-$) exhibited addition of 2 amu predicting

less double bond and they were tentatively identified as Dihydroxy-octadecadienoic acid and Dihydroxy-octadecenoic acid [12] [13].
Peaks 31 (*m/z* 293 $C_{18}H_{29}O_3^-$) and 32 (*m/z* 295 $C_{18}H_{31}O_3^-$) exhibited a mass difference of 2 amu suggesting less double bond and they were tentatively identified as hydroxy- octadecatrienoic acid and hydroxy-octadecadienoic acid [11].

3.2 Anticoagulant activity

In the current study, the effects of ethanolic extract of flowers, roots and leaves of *L. camara* L. as anticoagulant agent was investigated using two concentrations (1 and 0.5 mg/ml) for each extract. The anticoagulant activity was evaluated by measuring the prothrombin time (PT) using normal citrated human plasma. Heparin was used as positive control while DMSO was used as negative control. The results were illustrated in table (2) and figures (2), (3), (4) and (5).

Both concentrations of ethanolic extract of flowers and roots (1 and 0.5 mg/ml) showed significant differences $P< 0.0005$ and $P< 0.05$ (one-way ANOVA) respectively. While both concentrations of leaves did not exhibit any significant differences $P>0.05$ (one-way ANOVA) compared to control. The flower extract increased the clot time in relative to the control more than that done by the root extract.

Table (2): Effect of *Lantana camara* L. extracts (flowers, roots and leaves) on prothrombin time (PT) of human plasma.

Groups	PT(second)
Saline	14.1±0.46
Heparin	111.8^{a}± 5.0
F_1	$21.7^{a,b}$± 3.0
F_2	$20.1^{a,b}$± 2.9
F_3	15.9^{b}± 1.1
F_4	15.1^{b}± 1.1
R_1	$21.5^{a,b}$± 2.8
R_2	$19.8^{a,b}$± 3.1
R_3	15.1^{b}± 1.5
R_4	14.6^{b}± 1.3
L_1	16.9^{b}± 2.4
L_2	16.7^{b}± 3.3
L_3	15.6^{b}± 1.9
L_4	15.5^{b}± 1.8

a: Significantly different from control; b: Significantly different from heparin
F_1: Flower extract (1 mg/ml), F_2: Flower extract (0.5 mg/ml), F_3: Flower extract (0.25 mg/ml), F_4: Flower extract (0.125 mg/ml)
R_1: Root extract (1 mg/ml), R_2: Root extract (0.5 mg/ml), R_3: Root extract (0.25 mg/ml), R_4: Root extract (0.125 mg/ml)
L_1: Leaves extract (1 mg/ml), L_2: Leaves extract (0.5 mg/ml), L_3: Leaves extract (0.25 mg/ml), L_4: Leaves extract (0.125 mg/ml)

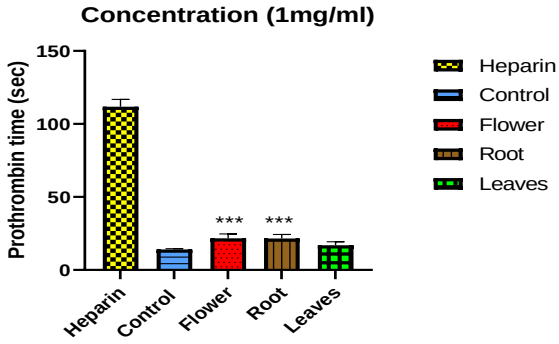

Fig. (2): Anticoagulant activity of *L. camara* L extract (1 mg/ml).

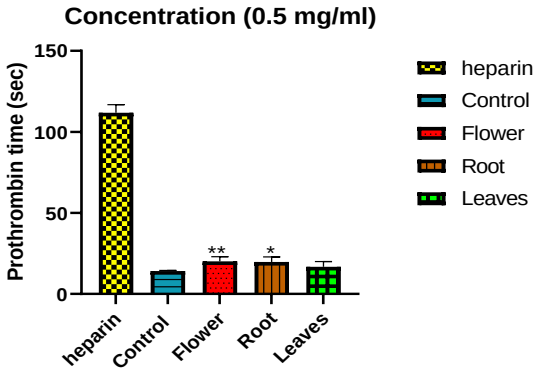

Fig. (3): Anticoagulant activity of *L. camara* L extract (0.5 mg/ml).

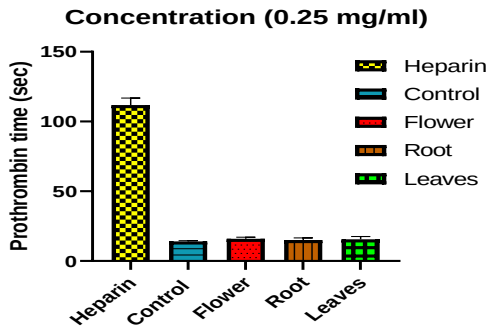

Fig. (4): Anticoagulant activity of L. camara L extract (0.25 mg/ml).

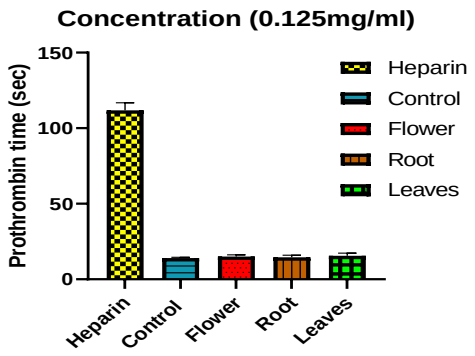

Fig. (5): Anticoagulant activity of *L. camara* L extract (0.125 mg/ml).

4. Discussion:

Phenolic acids are secondary metabolites distributed widely in plants[3]. They reported to inhibit growth of pathogens and cancer cells. They also possess strong antioxidant, and their presence as glycosides in vegetables, fruits or flowers is believed to protect against the damaging effect caused by UV, fungus, or other pathogens[27][28].

The identification of phenolic acids by mass spectrometry using ESI-in negative mode can provide high specificity and sensitivity whereby the structure of unknown phenolic acid derivatives can be assessed[29][30][7].

The combination of UV-Vis absorptions between 210-278 nm and mass detection of each peak in the LC-DAD-ESI-Ms and detection of the molecular mass of the phenolic acid, and type of sugar (s), attached can be helpful and important advantage to the identification process. Based on the $(M-H)^-$ MS^2 of the precursor ion and sequential loss of sugar moieties after elimination of water and CO_2, information about the gross structure can be deduced.

Comparison of the absorption spectrum or reported mass data about the compound in the literature can conclusively lead to identification of the structure in each peak with high degree of accuracy after analysis of MS^2 and MS^3.

The LC/MS profiling evidenced the existence of thirty-two bioactive phenolic, non-phenolic as well as oxygenated fatty acids. Derivatives of benzoic acid exist as free acids, in glycoside forms, and linked also with other acids. Acids and dimers of protocatechuic, syringic, and gallic which predominate are mostly bound with sugars or other acids like caffeic. Cinnamic acid derivatives are present linked to sugars or other acids and exemplified by sinapic acid, coumaric, ferulic and caffeic acids and their dimers). Non-phenolic acids were also tentatively characterized.

The PT test indicates the deficiencies in factors II, V, VII, and X involved in clot formation process. Thus, the present results suggest that ethanolic extract of flowers and roots inhibit preferentially extrinsic pathways of coagulation.

The highest anticoagulant activity was reported for ethanolic extract of flowers followed by that of roots while leaves ethanolic extract did not show any significant activity.

The anticoagulant activity of flowers of *L. camara* L. may be attributed to their high phenolic content as many studies have reported the anticoagulant activity of polyphenols. [14] reported that polyphenolics played a role in prolonging blood clotting in the intrinsic pathway. Phenolic acids and polyhydroxy phenolic compounds were also investigated for their possible anticoagulant activity in both *in vivo* and *in vitro* assays. Obtained results showed significant prolongation of bleeding time, coagulation time, PT, activated partial thromboplastin time (aPTT), and thrombin time (TT) [15].

A previous study revealed the thrombin inhibitory activity of leaves methanolic extract of *L. camara* L. Thrombin activity was measured as a function of clot formation from fibrinogen through a turbidometric assay. The activity was attributed to 5,5-trans-fused cyclic lactone-containing euphane triterpenes [16]**.** The present study did not reveal any significant difference in prothrombin time so it suggests that leaves of *L. camara* L. may affect the coagulation process in a different mechanism other than that measured in PT test.

6. Conclusion:

In conclusion, the obtained experimental data strongly support the view that the flower of *L. camara* can be used as anticoagulant agent. Its activity can be attributed to its phenolic constituents. Reaching these positive findings seen in this article, it is recommended that preclinical studies on the plant should be initiated.

References:

[1] Ezzat MI, El Gendy SN, Saad AS, Abdo WS, EL Sayed AM, Elmotayam AK. Secondary metabolites from Lantana camara L. flowers extract exhibit in vivo anti-urolithiatic activity in adult Wistar albino rats. Nat Prod Res 2020;0:1–4. https://doi.org/10.1080/14786419.2020.1853726.

[2] Jagdish S, Bhutani KK. Biological Action of Lantadene C , A New Hepatotoxicant from Lantana camara var . aculeata 1992;7:73–9.

[3] Khadem S, Marles RJ. Monocyclic Phenolic Acids; Hydroxy- and Polyhydroxybenzoic Acids: Occurrence and Recent Bioactivity Studies. Molecules 2010;15:7985–8005. https://doi.org/10.3390/molecules15117985.

[4] Robbins RJ. Phenolic Acids in Foods : An Overview of Analytical Methodology Review Phenolic Acids in Foods : An Overview of Analytical Methodology. Agric Food Chem 2003;51:2866–87. https://doi.org/10.1021/jf026182t.

[5] Félix-silva J, Souza T, Barros R, Camara G, Cabral B, Silva-júnior AA, et al. In vitro anticoagulant and antioxidant activities of Jatropha gossypiifolia L . (Euphorbiaceae) leaves aiming therapeutical applications. BMC Complement Altern Med 2014;14:1–13.

[6] Dakiche H, Khali M, Abu-el-Haija AK, Al-Maaytah A, Al-Balas QA. Biological activities and phenolic contents of Argania spinosa L (Sapotaceae) leaf extract. Trop J Pharm Res 2016;15:2563–70. https://doi.org/10.4314/tjpr.v15i12.5.

[7] Sobeh M, Mahmoud MF, Petruk G, Rezq S, Ashour ML, Youssef FS, et al. Syzygium aqueum: A polyphenol- rich leaf extract exhibits antioxidant, hepatoprotective, pain-killing and anti-inflammatory activities in animal models. Front Pharmacol 2018;9:1–14. https://doi.org/10.3389/fphar.2018.00566.

[8] Wang C, Zhang X, Guo Y, Wang C, Li G, Xu J. Phenolic compounds from Origanum vulgare and their antioxidant and antiviral activities Phenolic compounds from Origanum vulgare and their antioxidant and antiviral activities. FOOD Chem 2014;152:300–6. https://doi.org/10.1016/j.foodchem.2013.11.153.

[9] Muzaffar S, Bose C, Banerji A, Nair BG. Anacardic acid induces apoptosis-like cell death in the rice blast fungus Magnaporthe oryzae. Appl Microb Biotechnol 2016;100:323–35. https://doi.org/10.1007/s00253-015-6915-4.

[10] Wang S, Gao Z, Chen X, Lian X, Zhu H. The anticoagulant ability of ferulic acid and its applications for improving the blood compatibility of silk fibroin. Biomed Mater 2008;3:1–5. https://doi.org/10.1088/1748-6041/3/4/044106.

[11] Luo X, Du C, Cheng H, Chen J, Lin C. Study on the Anticoagulant or Procoagulant Activities of Type II Phenolic Acid Derivatives. Molecules 2017;22:1–16. https://doi.org/10.3390/molecules22122047.

[12] Wei J, Liu K, Lin F, He C, Luo X, Zou J, et al. Synthesis, and antioxidant, thrombin-inhibitory, and anticancer activities of hydroxybenzamide derivatives. Appl Biol Chem 2016;59:271–81. https://doi.org/10.1007/s13765-016-0157-8.

[13] Liang L, Zhao W, Meng F, Liu L, Lin C. Synthesis of Amide Compounds of Ferulic Acid and Their Association with Bovine Serum Albumin. Spectrosc Lett 2013;46:544–53. https://doi.org/10.1080/00387010.2012.729550.

[14] Barrett YC, Wang Z, Knabb RM. A Novel Prothrombin Time Assay for Assessing the Anticoagulant Activity of Oral Factor Xa Inhibitors. Clin Appl Thromb 2013;19:522–8. https://doi.org/10.1177/1076029612441859.

[15] Irhimeh MR, Fitton JH, Lowenthal RM. Pilot clinical study to evaluate the anticoagulant activity of fucoidan. Blood Coagul Fibrinolysis 2009;00:1–5. https://doi.org/10.1097/MBC.0b013e32833135fe.

[16] Hossain M, Brunton N, Martin-diana AB, Barry-ryan C. Characterization of Phenolics Composition in Lamiaceae Spices by LC-ESI-MS / MS. Agric Food Chem 2010;58:10576–81. https://doi.org/10.1021/jf102042g.

[17] Sun J, Liang F, Bin Y, Li P, Duan C. Screening Non-colored Phenolics in Red Wines using Liquid Chromatography/Ultraviolet and Mass Spectrometry/Mass Spectrometry Libraries. Molecules 2007;12:679–93.

[18] Allen F, Greiner R, Wishart D. Competitive fragmentation modeling of ESI-MS/MS spectra for putative metabolite identification. Metabolomics 2015;11:98–110. https://doi.org/10.1007/s11306-014-0676-4.

[19] Chen HJ, Inbaraj BS, Chen BH. Determination of phenolic acids and flavonoids in Taraxacum formosanum kitam by liquid chromatography-tandem mass spectrometry coupled with a post-column derivatization technique. Int J Mol Sci 2012;13:260–85. https://doi.org/10.3390/ijms13010260.

[20] Ana Plazonić, Bucar F, Maleŝ Željan, Mornar A, Nigović B, Kujundžić N. Identification and quantification of flavonoids and phenolic acids in burr parsley (caucalis platycarpos L.), using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry. Molecules 2009;14:2466–90. https://doi.org/10.3390/molecules14072466.

[21] Bystrom LM, Lewis BA, Brown DL, Rodriguez E, Obendorf RL. Characterisation of phenolics by LC-UV/Vis, LC-MS/MS and sugars by GC in Melicoccus bijugatus Jacq. “Montgomery” fruits. Food Chem 2008;111:1017–24. https://doi.org/10.1016/j.foodchem.2008.04.058.

[22] Brent LC, Reiner JL, Dickerson RR, Sander LC. Method for characterization of low molecular weight organic acids in atmospheric aerosols using ion chromatography mass spectrometry. Anal Chem 2014;86:7328–36. https://doi.org/10.1021/ac403937e.

[23] Farag S, Makboul M, Mohamed N, Attia A. Investigation of essential oil and biological activities of Lantana montevidensis (Spreng.) Briq. cultivated in Egypt. J Nat Pharm 2013;4:13. https://doi.org/10.4103/2229-5119.110342.

[24] Farag MA, El-Ahmady SH, Elian FS,

Wessjohann LA. Metabolomics driven analysis of artichoke leaf and its commercial products via UHPLC-q-TOF-MS and chemometrics. Phytochemistry 2013;95:177–87. https://doi.org/10.1016/j.phytochem.2013.07.003.

[25] Otify A, George C, Elsayed A, Farag MA. Mechanistic evidence of Passiflora edulis (Passifloraceae) anxiolytic activity in relation to its metabolite fingerprint as revealed via LC-MS and chemometrics. Food Funct 2015;6:3807–17. https://doi.org/10.1039/C5FO00875A.

[26] Farag MA, El Sayed AM, El Banna A, Ruehmann S. Metabolomics reveals distinct methylation reaction in MeJA elicited Nigella sativa callus via UPLC–MS and chemometrics. Plant Cell Tissue Organ Cult 2015;122:453–63. https://doi.org/10.1007/s11240-015-0782-7.

[27] Koolen HHF, da Silva FMA, Gozzo FC, de Souza AQL, de Souza ADL. Antioxidant, antimicrobial activities and characterization of phenolic compounds from buriti (Mauritia flexuosa L. f.) by UPLC-ESI-MS/MS. Food Res Int 2013;51:467–73. https://doi.org/10.1016/j.foodres.2013.01.039.

[28] Preethi KC, Kuttan G, Kuttan R, Preethi KC, Kuttan G, Kuttan R. Antioxidant Potential of an Extract of Calendula officinalis . Flowers in Vitro . and in Vivo . Antioxidant Potential of an Extract of Calendula officinalis Flowers in Vitro and in Vivo. Pharm Biol 2006;44:691–7. https://doi.org/10.1080/13880200601009149.

[29] Said R Ben, Hamed AI, Mahalel UA, Al-Ayed AS, Kowalczyk M, Moldoch J, et al. Tentative characterization of polyphenolic compounds in the male flowers of Phoenix dactylifera by liquid chromatography coupled with mass spectrometry and DFT. Int J Mol Sci 2017;18:1–18. https://doi.org/10.3390/ijms18030512.

[30] Mertz C, Cheynier V, Günata Z, Brat P. Analysis of phenolic compounds in two blackberry species (Rubus glaucus and Rubus adenotrichus) by high-performance liquid chromatography with diode array detection and electrospray ion trap mass spectrometry. J Agric Food Chem 2007;55:8616–24. https://doi.org/10.1021/jf071475d.

[31] Khoo LT, Abas F, Abdullah JO, Rahayu E, Tohit M, Hamid M. Anticoagulant Activity of Polyphenolic-Polysaccharides Isolated from Melastoma malabathricum L . Evidence-Based Complement Altern Med 2014;2014:1–9.

[32] Zong S, Ji J, Li J, Yang Q, Ye M. Physicochemical properties and anticoagulant activity of polyphenols derived from Lachnum singerianum. J Food Drug Anal 2016:4–11. https://doi.org/10.1016/j.jfda.2016.08.011.

[33] O'Neill MJ, Lewis JA, Noble HM, Holland S, Mansat C, Farthing JE, et al. Isolation of translactone-containing triterpenes with thrombin inhibitory activities from the leaves of Lantana camara. J Nat Prod 1998;61:1328–31. https://doi.org/10.1021/np970464j.