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HPTLC Fingerprint Profile of Triterpenes of *Lamium amplexicaule* Benth. and *Ajuga iva* L. (Lamiaceae) Monitored with Screening of their Anti-inflammatory Effect

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Abstract
Fingerprint profiles of triterpene contents of the methanol extracts of *Lamium amplexicaule* Benth. and *Ajuga iva* L. (Lamiaceae) were carried out using HPTLC where each herb was separately extracted continuously with methanol in soxhlets.
Fingerprinting was performed using specific solvent systems followed by densitometric scanning under ultraviolet using anisaldehyde sulphuric acid reagent, the gained triterpene profiles of both herbs were used as chemotaxonomic markers which exhibit significant value in differentiating these species from closely related adulterants.
The prepared methanol extracts were screened for their anti-inflammatory effects using carrageenan induced rat paw edema technique where pre-treatment with both extracts at dose levels 50 - 200 mg/kg significantly prevented increase in volume of paw edema in dose dependent manner, the maximal effect was observed at 200 mg/kg which was comparable to diclofenac potassium (20 mg/kg, orally).

Keywords: *Lamium amplexicaule* Benth, *Ajuga iva* L., Lamiaceae, Triterpenes, HPTLC, Fingerprinting, Anti-inflammatory

Introduction
Lamium and *Ajuga* are two genera belonging to family Lamiaceae composed of almost 40 and 301 species of flowering plants respectively, they are herbaceous plants native to Europe, Asia, Northern Africa, and are now widely naturalized across much of the temperate world [1, 2].
Genus *Lamium* has been found to possess significant pharmacological effects including antioxidant, free radical scavenging, antiproliferative, anti-inflammatory, antinociceptive, bacteriostatic, cytotoxic, antispasmodic and tyrosine inhibiting activities [3-8] while *Ajuga* species are widely used for the treatment of hypertension, hyperglycemia, pneumonia, acute and chronic pharyngitis [9-11], in addition, *Ajuga* has been used in Iranian traditional medicine for the treatment of joint pain, gout, and jaundice [12].
Phytochemical investigation *Lamium* species revealed the existence of several iridoids and secoiridoids, phenylpropanoids, flavonoids, anthocyanins, phytoecdysteroids, betaines, benzoxazinoids, terpenes, and megastigmen compounds as well as essential oils [13-20] while that of *Ajuga* species revealed the existence of phytoecdysteroids, neo-clerodane-diterpenes and diterpenoids, triterpenes, sterols, anthocyanin glycosides, iridoid glycosides, withanolides, flavonoids, triglycerides and essential oils [21-28].
Triterpenes are a class of natural products present in all organisms, especially in plants exhibiting unique important biological and pharmacological activities, including anti-inflammatory, antimicrobial, antiviral, cytotoxic and cardiovascular effects [29-33], they are regularly isolated as active substances in plants of the Lamiaceae family indicating the importance of these substances as nutraceuticals [34, 35].
Medicinal plant extracts are used in their aqueous or alcoholic forms in manufacturing herbal formulations and phytochemical profile and fingerprinting of the plant can always be used as an effective quality authentication tool which can be carried out through chromatography for ascertaining the presence of the essential chemical constituents of a medicinal plant as well as detection of their presence in a herbal preparations [36, 37].
HPTLC is being used for fingerprint profiling of medicinal plant extracts since long time ago [38, 39], the gained profiles have been proved to be effective tools in differentiating closely

related species and detecting adulteration and substitution in raw drugs [40]

This study was conducted to a fingerprint triterpene compounds in the methanol extracts of *Lamium amplexicaule* Benth. and *Ajuga iva* L. using HPTLC as well as screening of their anti-inflammatory effects.

Material and methods

Plant Materials

Aerial parts of *Lamium amplexicaule* Benth. and *Ajuga iva* L. were collected, from Tanta region Egypt during flowering stage at April 2014 and kindly identified by Dr. Moneer Abd El-Ghany Prof. of Plant Taxonomy, Faculty of Science, Cairo University, washed, air dried in shade, powdered and kept in tightly closed amber colored glass containers protected from light at temperature.

Voucher specimens are kept in a herbarium, Pharmacognosy, Department, Faculty of Pharmacy Al -Azhar University, Cairo, Egypt.

Extraction

50 g of each dried powdered plant under investigation was extracted separately by Soxhlet for 8-12 h with methanol, after filtration, extracts were concentrated under vacuum then washed within hexane until the chlorophyll was completely removed; the washed methanol extracts were filtered and used for study.

HPTLC fingerprinting

100 µl of each extract were taken and diluted to one ml, then subjected to HPTLC quantitative phytochemical characterization [41, 42] using CAMAG HPTLC system equipped with Linomat 5 applicator, TLC scanner 3 and WIN CATS software.

High-Performance Thin-Layer Chromatography was performed on silica gel 60F254 (10 cm× 10 cm; Merck) where the methanol extracts of the selected plants (10 mg/ml) were subjected to analysis after spotting on a silica gel 60F254 TLC plate, the plate was air dried and then developed by using Toluene: Chloroform: Ethanol (5:4:1) as mobile phase in a CAMAG twin-trough glass chamber (20x10x4) previously saturated with mobile phase vapor for 20 min, after development, plate was dried at 105 ºC for 15 min and then it was scanned using Scanner 3 (CAMAG, Switzerland) at 366 nm using Win CATS software. Chromatograms were evaluated before and after spraying with Anisaldehyde/sulphuric acid reagent.

Anti-inflammatory activity

Adult albino rats of either sex weighing 150-200 g purchased from The Animal House Laboratory, National research center, Cairo, Egypt were used for this study, they were housed in an environmentally control room, maintained at uniform light and temperature conditions of and provided with food and water *ad libitum*.

The anti-inflammatory activity of the prepared extracts was determined using carrageenan induced rat paw edema assay (43) where healthy rats were divided into ten groups - six rats each and were classified as follows;

Group 1; the negative control group received 1% (v/v) DMSO in water p.o. at a dose of 5 ml/kg.

Group 2; the positive control group was treated orally with the standard drug, diclofenac (20 mg/kg).

Group 3-6; test groups received the methanol extract of *Lamium amplexicaule* Benth. at doses of 50, 100, 150 and 200 mg/kg p.o.

Groups 7-10; test groups received the methanol extract of *Ajuga iva* L. at doses of 50, 100, 150 and 200 mg/kg p.o.

All the doses were administered 30 min before induction of edema by administering 0.1 ml of 1% w/v carrageenan in saline in sub plantar region of hind paw of each animal.

The degree of paw edema of all groups was measured using a plethysmometer (Ugo Basile, Italy) at 60, 120, 180 and 240 min after the administration of carrageenan.

Statistical studies

The results were subjected to two way ANOVA followed by dunnet's test, the data is deemed to be statistically significant if $p<0.05$.

Results and discussions

HPTLC technique is more precise when compared to TLC technique and thus the present study was carried out to gain comparative HPTLC profiles of triterpenes in the methanol extracts of two closely related genera of the same family [44]. In HPTLC technique, the chemical constituents in a chosen medicinal plant material from a characteristic fingerprint, representing the quantity of active constituents, in addition help in standardization of such mixtures in herbal drug formulations and market samples [44], the HPTLC densitometric scanning results observed in the present study are illustrated in table, 1, figures 1-4 which revealed that *Lamium amplexicaule* Benth. methanol extract showed definite twelve excellent bands, in solvent system Toluene: Chloroform: Ethanol (4:4:1), (Table1, Figure-1 A & B, Figure-2) when viewed in UV light at 254 nm, 366 nm before and after derivatization with anisaldehyde sulphuric acid reagent, good separation of constituents with different Rf values were observed while the methanol extract of *Ajuga iva* L. also showed 11 bands in the same solvent system with different Rf values (Table1, Figure-3 A & B, Figure-4) concluding that both plants showed same triterpene compounds in the Rf values 0.13, 0.20, 0.35, 0.43 and 0.78 where bandwidth and peak area was the maximum in the band with Rf 0.06 for *L amplexicaule Benth*. and minimum with Rf 0.86 (Figure-2) and *Ajuga iva* L. showed the maximum peak area in the Rf 0.08 and least width with Rf 0.78 (Figure-4).

Table 1: Comparative chart of Peak, HR_f, and peak characters of methanol extracts of *Lamium amplexicaule* Benth. and *Ajuga iva* L.

Peak	*Lamium amplexicaule* Benth.			Peak	*Ajuga iva* L.		
	R_f	Max. height	Area		R_f	Max. height	Area
1	6	151.2	5209	1	6	172.0 4	4918.1
2	13	90.8	2702.5	2	13	94.6	4536.4
3	20	25.6	493.2	3	20	76.1	1445.5
4	27	25	670.5	4	27	73.1	2652.9
5	35	139	3610	5	35	83.7	2739.1
6	43	105	3101	6	43	136.4	3734.7
7	44	125.1	2667	7	44	70.8.	1880.6
8	49	41.7	1130	8	49	38.0	773.5
9	55	28	780	9	55	17.5	559.0
10	69	37	7840	10	69	12.5	381.3
11	72	83	2320	11	72	15.6	377.6
12	79	27	2470	12	79	--	--

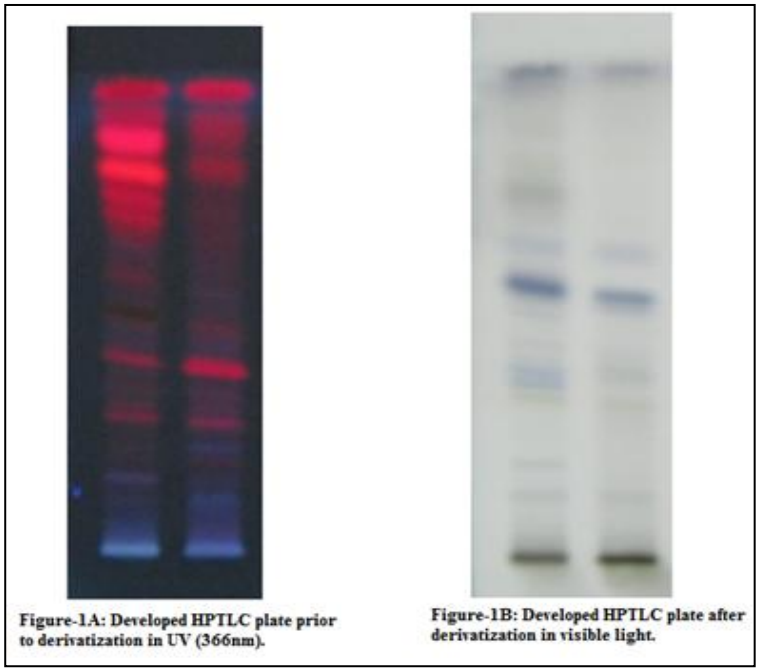

Fig 1: HPTLC Chromatogram of the methanol extract of *Lamium amplexicaule* Benth. (Lamiaceae), before and after derivatization using solvent system- Toluene: Chloroform: Ethanol (5:4:1).

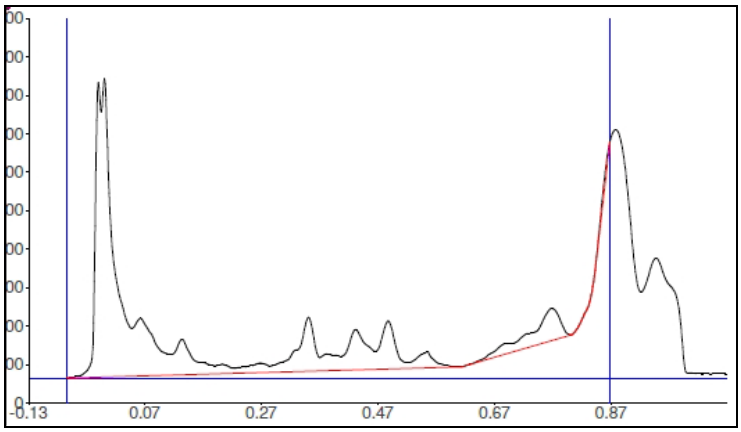

Fig 2: HPTLC Chromatogram of the methanol extract of *Lamium amplexicaule* Benth. (Lamiaceae).

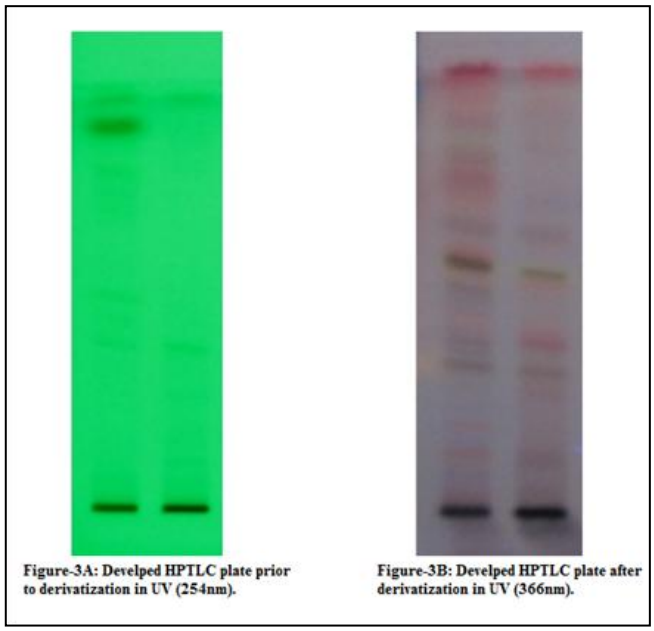

Fig 3: HPTLC Chromatogram of the methanol extract of *Ajuga iva* L. (Lamiaceae), before and after derivatization using solvent system- Toluene: Chloroform: Ethanol (5:4:1).

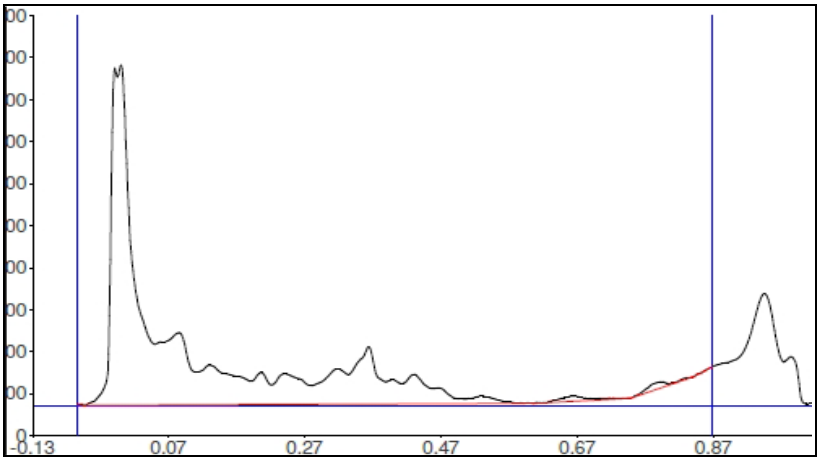

Fig 4: HPTLC Chromatogram of the methanol extract of *Ajuga iva* L. (Lamiaceae).

Carrageenan induced paw edema is a commonly used primary test for the screening of new anti-inflammatory agents and is believed to be biphasic, the first phase (1-2 h) is due to the release of histamine or serotonin and the second phase of edema is due to the release of prostaglandin [45].

The results of this study indicated that both the methanol extracts of *Ajuga iva* L. and *Lamium amplexicaule* Benth. Respectively caused significant reduction of carrageenan induced paw edema in rats in dose dependent manner (Table, 2) the mechanism of action may be due to inhibition of histamine, serotonin or prostaglandin synthesis through inhibition of prostaglandin synthetase within the hypothalamus [46].

Table 2: Effect of different doses of methanol extracts of *Lamium amplexicaule* Benth and *Ajuga iva* L. on carrageenan induced paw oedema in rats

aw volume (ml)	Negative control 5ml/kg	Positive control 20mg/kg	Doses of methanol extract of *Lamium amplexicaule* Benth. (mg/kg)				Doses of methanol extract of *Ajuga iva* L. (mg/kg)			
			50	100	150	200	50	100	150	200
60Min	0.45±0.05	0.23±0.05	0.26± 0.05*	0.24 ± 0.04*	0.20 ± 0.05*	0.19 ± 0.03	0.25 ± 0.06*	0.24 ± 0.01*	0.19 ± 0.03*	0.17 ± 0.03*
120 Min	0.68±0.01	0.35±0.07*	0.39 ± 0.06*	0.34 ± 0.05*	0.28 ± 0.06*	0.24 ± 0.03*	0.37 ± 0.07*	0.33 ± 0.05*	0.26 ± 0.05*	0.22 ± 0.02*
180 Min	0.79±0.04	0.43±0.02*	0.60 ±0.08*	0.48 ± 0.06*	0.43 ± 0.03*	0.41 ± 0.05*	0.57 ± 0.06*	0.44 ± 0.03*	0.40 ± 0.04*	0.39 ± 0.04*
240 Min	0.62±0.02	0.37±0.05*	0.54 ± 0.04	0.46 ± 0.05*	0.40 ± 0.06*	0.39 ± 0.03*	0.50 ± 0.04*	0.43 ± 0.04*	0.39 ± 0.03*	0.37 ± 0.05*

Values are expressed as mean ± standard error where (n=6); *p<0.05

Conclusion

The gained fingerprint profiles of triterpenes in *Lamium amplexicaule* Benth and *Ajuga iva* L. can be used for the identification and quality control of the extracts where both plants share common bands indicating their chemical similarity as two closely related genera belonging to the same family, in distinction they exhibited characteristic bands indicating their typical genera characters.

The extracts of the aforementioned medicinal plants justify further studies to isolate and characterize the active constituents; also it is important to develop a better understanding of their mode of therapeutic action and also for further medical applications.

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