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HPTLC finger print and anti-inflammatory activity of ethanolic extract of different *Maytenus* species grown in Kingdom of Saudi Arabia

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PEER REVIEW

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Comments

This is a good study in which the authors work to develop HPTLC finger print of ethanolic extract of *Maytenus obscura* and *Maytenus parviflora*. The final results are very interesting because of ethanolic extract of these plants having the potential compounds for anti-inflammatory activity.

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ABSTRACT

Objective: To evaluate and compare the anti-inflammatory activity and to develop HPTLC fingerprint profile of ethanolic extract of *Maytenus obscura* (*M. obscura*) and *Maytenus parviflora* (*M. parviflora*).

Methods: Preliminary phytochemical screening was done and HPTLC studies were carried out using CAMAG HPTLC system equipped with Linomat IV applicator, TLC scanner 3, Reprostar 3, CAMAG ADC 2 and WIN CATS-4 software. The anti-inflammatory activity was tested by injecting different groups of rats (6 each) with formalin in hind paw and measuring the edema volume before and 1 h after formalin injection. Control group received saline *i.p.* The extract treatment was injected *i.p.* with doses of 200 and 400 mg/kg 1 h before formalin administration. Indomethacin (30 mg/kg) was used as standard.

Results: Treatment of rats (*i.p.*) with *M. obscura* and *M. parviflora* in doses of 200 and 400 mg/kg inhibited significantly ($P < 0.05$) formalin-induced inflammation by 55.9%, 63.2% and 77.9%, 82.4%, respectively. Preliminary phytochemical studies were done which confirmed the presence of protein, lipid, carbohydrate, phenol, flavonoid, saponin, triterpenoid, alkaloid and anthraquinone. Chromatography was performed on glass-backed silica gel 60 F254 HPTLC plates with the solvent system: Toluene: ethylacetate: glacial acetic acid (5:2:0.1, v/v/v) as mobile phase. HPTLC finger printing of *M. obscura* revealed major 8 peaks with R_f values in the range of 0.27 to 0.77 and the *M. parviflora* revealed maximum 9 peaks with R_f values in the range of 0.17 to 0.76. The purity of sample was confirmed by comparing the absorption spectra at start, middle and end position of the band.

Conclusions: HPTLC of *M. parviflora* revealed 8 major spots and 9 spots for *M. obscura*. HPTLC finger printing of ethanolic extract of *M. obscura* and *M. parviflora* may become potential tool for checking authenticity of these species. It may help in quality control against adulterant and act as a biochemical marker for these medicinally important plants in the pharmaceutical industry and plant systematic studies. The anti-inflammatory potential of these plants also reveals its medicinal importance. It might be further explored for the isolation of phytoconstituents having anti-inflammatory potential.

KEYWORDS

Maytenus obscura, *Maytenus parviflora*, Phytochemical screening, Finger print, Standardization, HPTLC

1. Introduction

About 850–1300 species of the genus *Maytenus* is distributed worldwide, particularly in subtropical and

tropical regions, Africa, China, Brazil, Paraguay, Uruguay and Argentina and in southern regions of Saudi Arabia^[1]. *Maytenus* species are rich in triterpenes, diterpenes, sesquiterpene alkaloids and spermidine alkaloids^[2]. Other

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secondary metabolites produced by members of this genus include flavonoids and flavonoid glycosides; phenolic glucoside and agarofurans[3,4]. This genus is extensively investigated for bioactive compounds as they are widely used in folk medicine as antiseptic, antiasthmatic, fertility-regulating agents, antitumor, as well as antiulcer. *Maytenus aquifolium* is called “Espinheira-santa” (holly spines) and is marketed in the form of capsules, powders, dried leaves, fresh leaves, aqueous or aqueous-alcoholic preparations in countries of South America[5]. Various *Maytenus* plants are used alone or in combinations as sialogogue, antiasthmatic, antiseptic, vulnerary antitumor[1], antiulcer[5], antacid[6] and as beverage[5]. It is also used by Indian tribes and rural population in Paraguay as fertility regulating agents and to induce menses in women[5]. Many species have been used extensively as conventional medicament to treat various diseases. Phytoconstituents still play as alternative treatment in many under developed countries[7]. Standardization of plant materials is the primary requirement of the today’s health care system in order to check for quality of these products. Several pharmacopoeias containing monographs for the plant materials explains only the physicochemical standards and some non-specific analytical procedures. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for more accurate standardization of herbals[8]. High performance thin layer chromatography (HPTLC) is a priceless tool for decisive standardization. It can provide chromatographic fingerprints that can be visualized and stored as electronic images where they may be used as reference guides for future standardization. These days HPTLC has become a conventional analytical approach due to its benefit of low operation cost; high sample throughput and need for only minimum sample clean up. The main benefit of HPTLC is that many samples can be run simultaneously using a little volume of mobile phase thus reducing time and cost per analysis[9–13]. The aim of this study is to evaluate and compare the anti-inflammatory activity and to develop HPTLC fingerprint profile of ethanolic extract of *Maytenus obscura* (*M. obscura*) and *Maytenus parviflora* (*M. parviflora*).

2. Materials and methods

2.1. Plant material

M. obscura (A. Rich.) Cuf. and *M. parviflora* were collected from Abha, Kingdom of Saudi Arabia. The plant was collected and identified by Dr. Mohammad Atiqur Rahman; taxonomist of Medicinal, Aromatic and Poisonous

Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Saudi Arabia. Voucher specimens (14295, 13420, respectively) were deposited in the Herbarium of the College of Pharmacy, King Saud University, Saudi Arabia. Aerial parts were shade dried and coarsely powdered.

2.2. Preparation and extraction of plant material

The aerial parts of *M. obscura* and *M. parviflora* were air-dried and pulverized. Five hundred grams of the powdered material were exhaustively extracted with 90% ethanol by cold percolation for 72 h and subsequent filtration. Thereafter ethanolic extract of *M. obscura* and *M. parviflora* were concentrated under vacuum and finally left for complete drying. The yield of the *M. obscura* and *M. parviflora* were 14.2% (w/w) and 10.9 % (w/w) respectively.

2.3. Phytochemical screening

Preliminary phytochemical screening was done following the method of Kokate and Khandelwal[14,15].

2.4. HPTLC profile

2.4.1. Chromatographic method

HPTLC studies were carried out following the method of Harborne and Wagner *et al*[16,17]. The protocol for preparing sample solutions was optimized for high quality fingerprinting. The fingerprinting of *M. obscura* and *M. parviflora* were executed by spotting 5, 10, 15 and 20 μ L of suitably diluted sample solution of *M. obscura* and *M. parviflora* on a HPTLC plate. The plates were developed and scanned as same discussed above. The peak areas were recorded. Chromatography was performed on 20 cm \times 10 cm glass HPTLC plates precoated with 200 μ m layers of silica gel 60F254 (E. Merck, Darmstadt, Germany). Samples (5, 10, 15, 20 μ L) of each plant extract were applied as bands 6 mm wide and 8 mm apart by means of Camag (Muttentz, Switzerland) Linomat IV sample applicator equipped with a 25 μ L syringe which was programmed through WIN CATS software.

2.4.2. Solvent system development

A number of solvent systems were tried, but the best resolution was obtained with the solvent Toluene: ethylacetate (EA): glacial acetic acid (GAA): (5:2:0.1).

2.4.3. Detection of spots

The developed plate was dried at 100 $^{\circ}$ C in hot air oven for 3 min to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR

3) and images captured under UV light at 254 and 366 nm, respectively. The R_f values and finger print data were recorded by WIN CATS software.

2.5. Anti-inflammatory activity

Inflammation was induced in the plantar side of the left hind paws of male Wistar rats (weighing 100–150 g) by injecting 0.1 mL of 10% aqueous formalin as described by Fu *et al*[18]. The increase in the paw edema was recorded prior to and 60 min after formalin injection (or 2 h after treatment with extracts) using Hydro-Plethysmograph (Model 7150, Ugo, Basile, Haly). Instead of the treatment (extracts) (in the treated group) the Control group: was administered (*i.p.*) 0.1 mL of 10% aqueous formalin and the Standard group was given *i.p.* injection of Indomethacin (30 mg/kg)[19]. Treated group: The anti-inflammatory activity of the *M. obscura* and *M. parviflora* (200 and 400 mg/kg) were tested by injecting the treatment (*M. obscura* and *M. parviflora*) solubilized in suitable vehicle as described above 60 min (*i.p.*) before formalin injection. Then the procedure was continued as outlined above. Effect of the *M. obscura* and *M. parviflora* on the formalin-induced paw volume was recorded (2 h after extract treatment or 1 h after formalin injection) as percentage inhibition relative to the control untreated group and were compared to the standard group. Change in volume of edema was quantified using the following formula:

$$(L_2 - R_2) - (L_1 - R_1)$$

Where, L_1 : Left paw volume before injection of formalin.
 R_1 : Right paw volume before injecting left paw with formalin.
 L_2 : Left paw volume 1 hr after injecting formalin and 2 h after treatment.
 R_2 : Right paw volume 1 h after formalin injection in the left paw and 2 h after treatment.

2.6. Statistical analysis

Results were analyzed using One-way ANOVA and Dunnett test as post hoc test (for more than 2 groups) or Kruskal's wallis ANOVA with Dunn's test as post hoc test (unless otherwise stated) and presented as mean±SEM. Only results with $P \leq 0.05$ were regarded as significant[20].

3. Results

3.1. Phytochemical screening of *M. obscura* and *M. parviflora*

A preliminary phytochemical screening of *M. obscura* and *M. parviflora* yielded the presence of protein, lipid, carbohydrate, reducing sugar, phenol, tannin, flavonoid, saponin, triterpenoid, alkaloid and quinone (Table 1).

Table 1

Phytochemical contents of *M. obscura* and *M. parviflora*.

Chemical constituents	<i>M. obscura</i>	<i>M. parviflora</i>
Protein	+	+
Lipid	+	+
Carbohydrate	+	+
Phenol	++	++
Flavonoid	+++	+++
Saponin	++	++
Triterpenoid	+++	+++
Alkaloid	+++	++
Anthraquinone	++	++

3.2. HPTLC fingerprinting of *M. obscura* and *M. parviflora*

Table 2 and 3 show HPTLC fingerprinting of *M. obscura* and *M. parviflora*, respectively, which revealed several peaks. HPTLC profile of *M. obscura* and *M. parviflora* under UV 366 and 254 nm was recorded in the Figures 1 and 2, respectively. The corresponding HPTLC chromatograms were presented in Figures 3–10. The *M. obscura* revealed 5, 8, 8 and 8 spots with R_f values in the range of 0.27 to 0.77 for 5, 10, 15 and 20 μ L application volume respectively (Table 2 and Figures 3–6). However *M. parviflora* revealed 6, 9, 9 and 9 spots with R_f values in the range of 0.17 to 0.76 for 5, 10, 15 and 20 μ L application volume respectively (Table 3 and Figures 7–10). The purity of the sample was confirmed by comparing the absorption spectra at start, middle and end position of the band.

Table 2

HPTLC profile of the *M. obscura*.

VM	Peak	R_f	Height Area
5 μ L	1	0.27	274.8
	2	0.32	702.0
	3	0.48	1324.6
	4	0.67	685.1
	5	0.72	1568.6
10 μ L	1	0.27	854.2
	2	0.32	1360.3
	3	0.40	574.6
	4	0.48	2247.8
	5	0.59	367.9
	6	0.68	827.4
	7	0.71	1353.3
	8	0.77	693.5
15 μ L	1	0.27	837.9
	2	0.32	1950.0
	3	0.39	664.2
	4	0.48	3118.0
	5	0.59	501.6
	6	0.67	910.7
	7	0.71	1646.0
	8	0.77	733.3
20 μ L	1	0.27	974.2
	2	0.32	2277.2
	3	0.39	714.5
	4	0.47	3707.9
	5	0.58	641.0
	6	0.66	933.0
	7	0.70	1946.8
	8	0.76	952.5

VM: Volume of *M. obscura* applied.

Table 3

HPTLC profile of the *M. parviflora*.

VMP	Peak Area	R_f	Height
5 μ L	1	0.24	213.2
	2	0.32	2105.9
	3	0.53	713.6
	4	0.66	1423.6
	5	0.70	2360.9
	6	0.76	1302.2
10 μ L	1	0.17	212.1
	2	0.26	1053.0
	3	0.32	3066.6
	4	0.35	1446.4
	5	0.43	879.8
	6	0.52	1256.7
	7	0.65	3062.7
	8	0.69	4783.4
	9	0.76	2760.0
15 μ L	1	0.17	275.0
	2	0.26	1300.7
	3	0.31	3817.6
	4	0.37	2027.8
	5	0.42	1152.4
	6	0.52	1735.1
	7	0.64	4264.9
	8	0.68	6344.0
	9	0.75	3929.7
20 μ L	1	0.17	409.8
	2	0.26	1718.6
	3	0.31	4646.8
	4	0.36	2763.7
	5	0.42	1321.9
	6	0.51	2031.0
	7	0.64	4580.1
	8	0.68	7839.5
	9	0.75	4442.9

VMP: Volume of *M. parviflora* applied

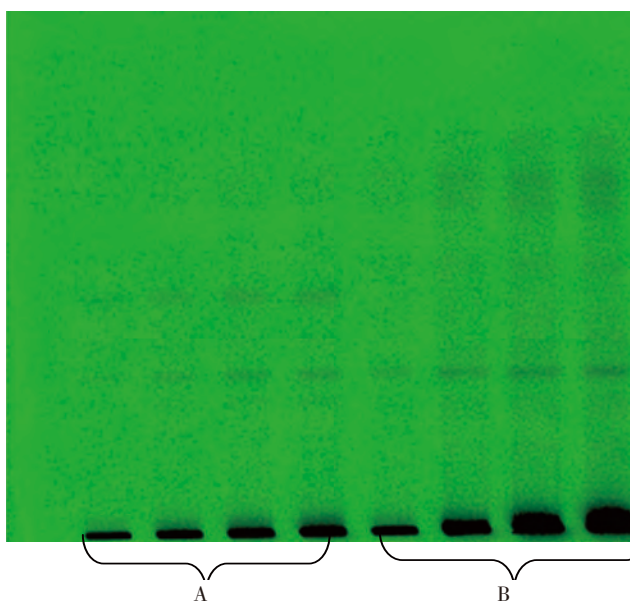


Figure 1. A: HPTLC picture of the *M. obscura* at 5, 10, 15 and 20 μ L of sample application respectively at UV 254 nm; B: HPTLC picture of the *M. obscura* at 5, 10, 15 and 20 μ L of sample application respectively at UV 254 nm.

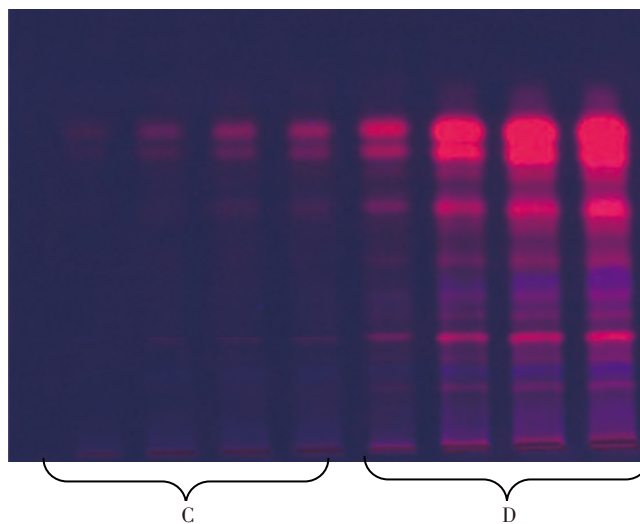


Figure 2. C: HPTLC picture of the *M. obscura* at 5, 10, 15 and 20 μ L of sample application respectively at 366 nm; D: HPTLC picture of *M. parviflora* at 5, 10, 15 and 20 μ L of sample application respectively at 366 nm.

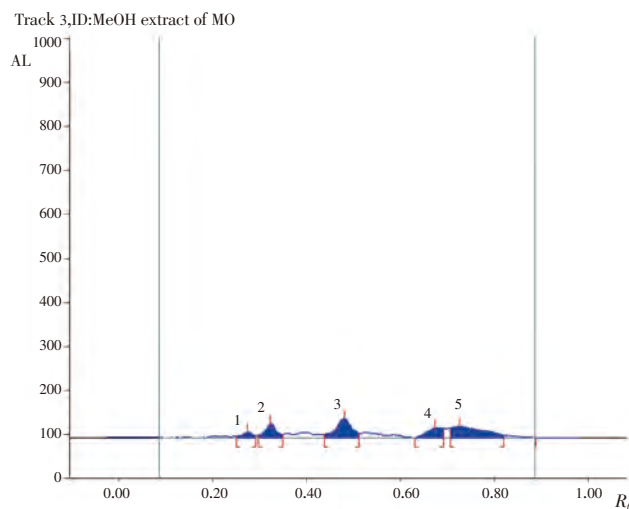


Figure 3. HPTLC chromatogram of *M. obscura* (5 μ L) showing different peaks of phytoconstituents.

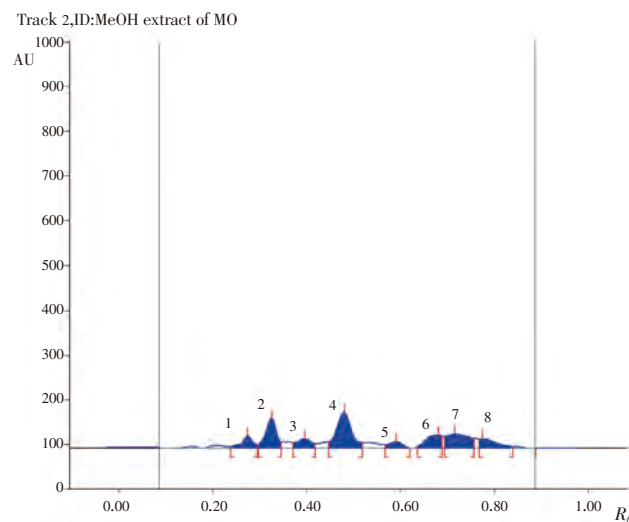


Figure 4. HPTLC chromatogram of *M. obscura* (10 μ L) showing different peaks of phytoconstituents.

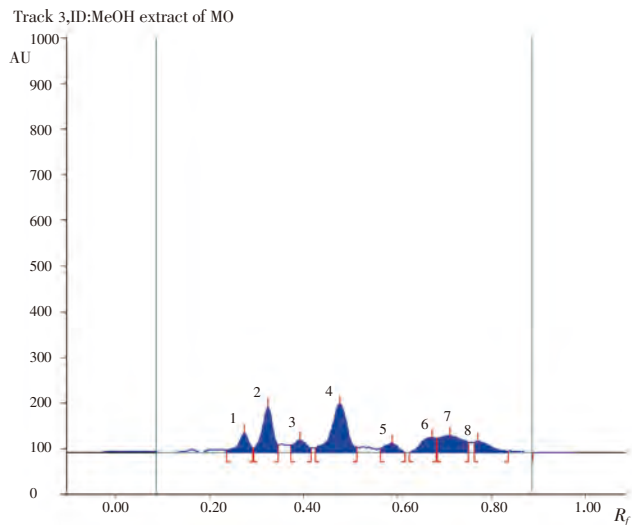


Figure 5. HPTLC chromatogram of *M. obscura* (15 µL) showing different peaks of phytoconstituents.

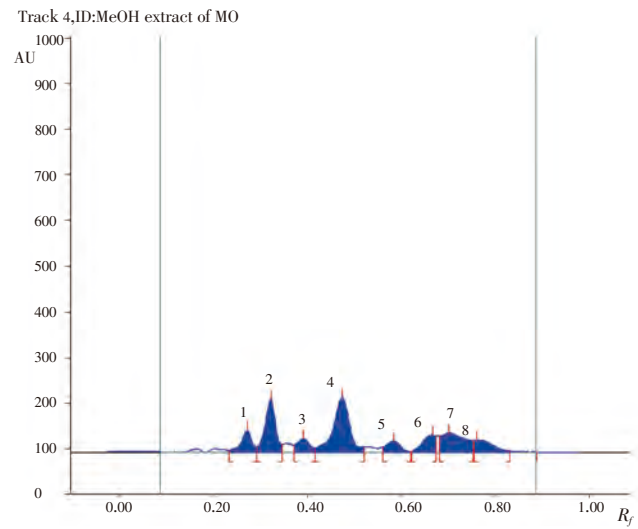


Figure 6. HPTLC chromatogram of *M. obscura* (20 µL) showing different peaks of phytoconstituents.

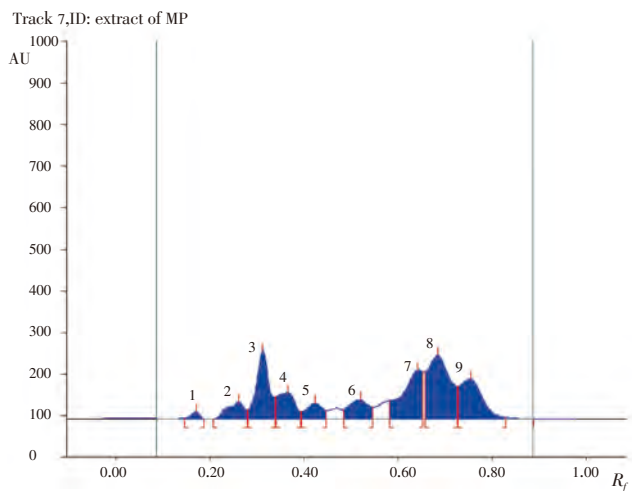


Figure 7. HPTLC chromatogram of *M. parviflora* (5 µL) showing different peaks of phytoconstituents.

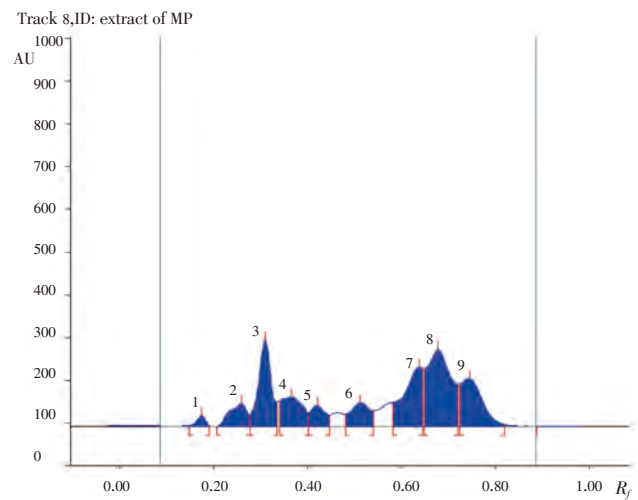


Figure 8. HPTLC chromatogram of *M. parviflora* (10 µL) showing different peaks of phytoconstituents.

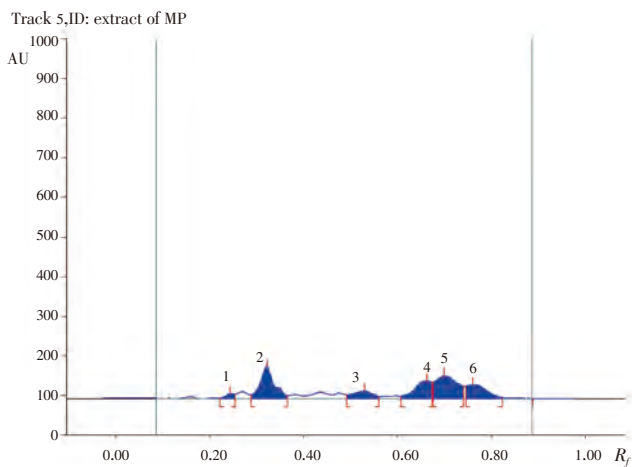


Figure 9. HPTLC chromatogram of *M. parviflora* (15 µL) showing different peaks of phytoconstituents.

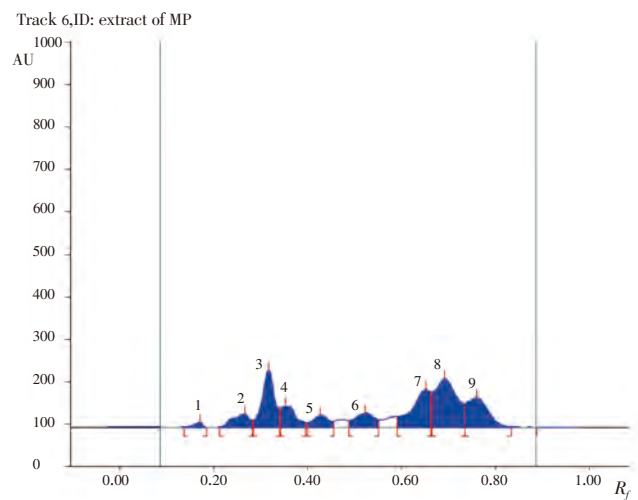


Figure 10. HPTLC chromatogram of *M. parviflora* (20 µL) showing different peaks of phytoconstituents.

3.3. Anti-inflammatory activity of *M. obscura* and *M. parviflora*.

Injection of formalin into a rat's hind paw resulted in swelling as early as 10 min after injection. This swelling reached a plateau by 30 min and was maintained for up to 240 min^[21]. Treatment of rats (*i.p.*) with *M. obscura* and *M. parviflora* in doses of 200 and 400 mg/kg inhibited significantly ($P < 0.05$, $n = 6$), (Table 4) formalin-induced inflammation by 55.9% and 77.9%, and 63.2% and 82.4%, respectively in a dose dependent manner.

Table 4

Effect of *M. obscura* and *M. parviflora* on formalin-induced inflammation in rats.

Treatment	Dose (mg/kg)	Paw edema volume (mL)	% inhibition
Control	0.1 mL (formalin)	0.68±0.06	0
<i>M. obscura</i>	200	0.30±0.01**	55.9
	400	0.25±0.03*	63.2
<i>M. parviflora</i>	200	0.15±0.01*	77.9
	400	0.12±0.03**	82.4
Indomethacine	30	0.10±0.02**	85.2

4. Discussion

The quality of herbal medicines is defined in terms of the content of its bioactive compounds. Hence, HPTLC fingerprint profile of herbal products is such an important and powerful procedure which has often been employed for the determination of bioactive components of the herbal medicine. HPTLC fingerprinting is proved to be a liner, precise, accurate method for herbal identification and can be used further in authentication and standardization of the medicinally important plant. Such finger printing is useful in quality control of herbal products and checking for the adulterant. Therefore, it can be useful for the evaluation of different marketed pharmaceutical preparations and plant systematic studies^[22]. *M. parviflora* and *M. obscura* possessed dose dependent and significant anti-inflammatory activities in doses of 200 and 400 mg/kg. HPTLC of *M. parviflora* revealed the presence of 5, 8, 8, 8 spots for 5, 10, 15 and 20 µL applications, respectively in R_f range 0.27–0.71 and *M. obscura* revealed 6, 9, 9, 9 spots for 5, 10, 15 and 20 µL applications, respectively, in the R_f range 0.17–0.76. Several peaks observed in this experiment indicate the diverse composition of *M. obscura* and *M. parviflora*. The marked anti-inflammatory activity showed by both *M. obscura* and *M. parviflora* revealed that both extracts are potential to be used in various inflammatory diseases after further safety studies. *M. parviflora* showed stronger anti-inflammatory activity compared to *M. obscura*. This

may indicated that phytoconstituents of *M. parviflora* were more potent than those of *M. obscura* in suppression of inflammation. Consequently this HPTLC fingerprinting may serve as reference to check the ability of other batches of ethanolic extracts of these plants to suppress inflammation. By exhaustive literature survey it is evident that there are no reports regarding fingerprinting analyses of ethanolic extract of *M. obscura* and *M. parviflora*. Hence, HPTLC fingerprinting is performed for the first time for these plants.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Different species of the genus *Maytenus* are extensively investigated for bioactive compound as they are widely used as antiseptic, antiasthmatic, fertility-regulating agents, antitumor, antiplasmodial, antimicrobial, as well as antiulcer. Many species belonging to the genus *Maytenus* have been used as a traditional medicine in the South America including Brazil used as an infusion or simply chewed for the treatment of pain and Paraguay as fertility regulating agents and to induce menses in women.

HPTLC is an important techniques for identification of different chemical compounds from medicinal plants. Nowadays, HPTLC fingerprint can be used further in authentication, standardization and quality control of herbal medicinal plants. The studies were carried out to evaluate and compare the anti-inflammatory activity and to develop HPTLC fingerprint profile of ethanolic extract of *M. obscura* and *M. parviflora*.

Research frontiers

The main cutting edge in the field of research in this paper is no reports regarding fingerprinting analyses of ethanolic extract and potential anti-inflammatory of *M. obscura* and *M. parviflora*.

Related reports

Anti-inflammatory studies have been performed in

species of *Maytenus senegalensis* by Sosa *et al.* (2007). This is the first report regarding HPTLC fingerprinting analyses of ethanolic extract and potential of anti-inflammatory activity of *M. obscura* and *M. parviflora*. The anti-inflammatory test used the same procedure as described by Fu *et al.*

Innovations & breakthroughs

The study showed that the *Maytenus* species have some phytochemicals, which are potential for anti-inflammatory activity.

Applications

This research report the potential of anti-inflammatory activity from *M. obscura* and *M. parviflora*. This is can be used for further explored for the isolation and characterization of bioactive compounds having anti-inflammatory potential.

Peer review

This is a good study in which the authors work to develop HPTLC finger print of ethanolic extract of *M. obscura* and *M. parviflora*. The final results are very interesting because of ethanolic extract of these plants having the potential compounds for anti-inflammatory activity.

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