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Anti-inflammatory activity and qualitative analysis of different extracts of *Maytenus obscura* (A. Rich.) Cuf. by high performance thin layer chromatography method

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PEER REVIEW

Peer reviewer

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Comments

The research represents advanced methodological qualitative analysis of complex matrix such as herbal medicine where only very limited information can be acquitted using traditional techniques. The manuscript is well written. It has been organized according to well documented researches. The materials and methods are written precisely and well referenced. The results are compatible with what the methods may generate. The results were discussed scientifically according to the results obtained.

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ABSTRACT

Objective: To perform aqueous ethanol soluble fraction (AESF) and dichloromethane extract of aerial parts of *Maytenus obscura* (A. Rich.) Cuf. using high performance thin layer chromatography (HPTLC) and to test anti-inflammatory activity of these extracts.

Methods: 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 were used. 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 later formalin injection. Control group received saline *i.p.* The extracts treatment was injected *i.p.* in doses of 100 and 200 mg/kg 1 h before formalin administration. Indomethacin (30 mg/kg) was used as standard.

Results: The results of preliminary phytochemical studies confirmed the presence of protein, lipid, carbohydrate, phenol, flavonoid, saponin, triterpenoid, alkaloid and anthraquinone in both extracts. Chromatography was performed on glass-backed silica gel 60 F254 HPTLC plates with the green solvents toluene: ethyacetate: glacial acetic acid (5:3:0.2, v/v/v) as mobile phase. HPTLC finger printing of AESF revealed major eight peaks with R_f values in the range of 0.28 to 0.80 and the dichloromethane revealed major 11 peaks with R_f values in the range of 0.12 to 0.76. The purity of sample was confirmed by comparing the absorption spectra at start, middle and end position of the band. Treatment of rats (*i.p.*) with AESF and dichloromethane in doses of 100 and 200 mg/kg inhibited singnificantly ($P<0.05$, $n=6$) formalin-induced inflammation by 50%, 55.9%, 45.5%, and 51.4%, respectively.

Conclusions: HPTLC finger printing of AESF and dichloromethane of *Maytenus obscura* revealed eight major spots for alcoholic extracts and nine major spots for dichloromethane extracts. These HPTLC profiles may be of great usefulness in the quality control of herbal products containing these extracts. The anti-inflammatory activity of both extracts also revealed the medicinal importance of these extracts. The plant can be further explored for the isolation of phytoconstituents having anti-inflammatory activity.

KEYWORDS

Maytenus obscura, Phytochemical screening, Finger print, Anti-inflammatory, HPTLC

1. Introduction

Maytenus obscura (A. Rich.) Cuf. (*M. obscura*) belongs to the genus *Maytenus* which is distributed worldwide, particularly in subtropical and tropical regions, Africa, China, Brazil, Paraguay, Uruguay and Argentina and in southern regions of Saudi Arabia[1]. The genus *Maytenus* is rich in triterpenes, diterpenes, sesquiterpene alkaloids and spermidine alkaloids[2]. Other secondary metabolites isolate from different species of the genus *Maytenus*,

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including flavonoids and flavonoid glycosides[3], phenolic glucoside and agarofurans[4]. Species belonging to this genus are extensively investigated for bioactive compounds as they are widely used in folk medicine such as antiseptic, antiasthmatic, fertility-regulating agents, antitumor and antiulcer[5,6]. Some *Maytenus* species are marketed in the form of capsules, powders, dried leaves, fresh leaves, aqueous or aqueous-alcoholic preparations[5]. Herbal drugs play major role in treatment of many diseases especially in undeveloped countries[7]. Standardization of plant materials used in traditional medicine is very important in today's health care system. Pharmacopoeial herbal profiles need to be updated to include the fingerprinting of herbal drugs generated by advanced technology. These modern methods describing the identification and quantification of active constituents in the plant material may be useful for complete standardization of herbals and its formulations[8]. High performance thin layer chromatography (HPTLC) can provide chromatographic fingerprints of herbal drugs that can be visualized, stored as electronic images and used as referene for quality control of herbs. Due to its low cost, high sample throughput and low sample cleanup, HPTLC may become one of standard quality testing techniques. HPTLC has the advantage of simultaneous analysis of large numbers of samples, 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 alcoholic and dichloromethane extract of aerial parts of *M. obscura*.

2. Materials and methods

2.1. Plant material

M. obscura was 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, College of Pharmacy, King Saud University, Saudi Arabia). Voucher specimens (14295, 13420 and 14309 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

About 1.3 kg of *M. obscura* was exhaustively extracted at room temperature with 90% ethanol (3×3 L). The ethanol extract was then concentrated under vacuum to yield 182.5 g black waxy residue. A total of 120 g was defatted with petroleum ether (4×200 mL). The remaining alcoholic extract was fractionated with CH_2Cl_2 (5×200 mL) to yield 13.2 g CH_2Cl_2 soluble fraction (SF) and 99.1 g aqueous ethanol soluble fraction (AESF).

2.3. Phytochemical screening

Preliminary phytochemical screening was done following the method of Kokate and Gokhale[14], and Khandelwal[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 dichloromethane and alcoholic extracts of *M. obscura* were executed by spotting 5, 10, 15, 20 μL of suitably diluted sample solution of the SF and AESF on a HPTLC plate. Chromatography was performed on 20 cm×10 cm glass HPTLC plates precoated with 200 μm layers of silica gel 60F254 (E. Merck, Darmstadt, Germany). Samples (5 μL, 10 μL, 15 μL, 20 μL) of each extract were applied as bands 6 mm wide and 8 mm apart by means of Camag (Muttenz, Switzerland) Linomat IV sample applicator equipped with a 25 μL syringe which was programmed through WIN CATS software. The developed plate was dried at 100 °C in hot air oven for 3 min. The plate was kept in photo-documentation chamber (CAMAG Reprostar 3) and captured the images under UV light at 254 and 366 nm, respectively. The R_f values and finger print data were recorded by WIN CATS software.

2.4.2. Solvent system development

A number of solvent systems were tried, but the best resolution was obtained in the solvent toluene: ethyacetate: glacial acetic acid (5:3:0.2).

2.4.3. Detection of spots

The developed plate was dried at 100 °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 SF and AESF (100 and 200 mg/kg) were tested by injecting the treatment (SF and AESF) 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 SF and AESF 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:

Change in volume of edema=$(L_2-R_2)-(L_1-R_1)$

Where L_1 is left paw volume before injection of formalin; R_1 is right paw volume before injecting left paw with formalin; L_2 is left paw volume 1 h after injecting formalin and 2 h after treatment; R_2 is 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 $P\leqslant 0.05$ were regarded as significant[20].

3. Results

3.1. Phytochemical screening of AESF and SF

A preliminary phytochemical screening of AESF and SF showed that both extracts contain protein, lipid, carbohydrate, reducing sugar, phenol, tannin, flavonoid, saponin, triterpenoid, alkaloid and quinone (Table 1).

Table 1

Phytochemical contents of AESF and SF.

Chemical constituents	AESF	SF
Protein	+	+
Lipid	+	++
Carbohydrate	+	+
Phenol	++	++
Flavonoid	+++	+++
Saponin	++	++
Triterpenoid	+++	+++
Alkaloid	++	++
Anthraquinone	++	+

3.2 HPTLC fingerprinting of AESF and SF

Tables 2 and 3 show HPTLC fingerprinting of AESF and SF, respectively, which revealed several peaks.

Table 2

HPTLC profile of the AESF.

Volume of AESF applied	Peak	R_f	Height area
	1	0.30	337.3
	2	0.35	810.1
5 μL	3	0.51	1638.9
	4	0.63	247.8
	5	0.73	772.6
	6	0.77	2139.4
	1	0.29	599.3
	2	0.35	1367.2
	3	0.41	452.0
10 μL	4	0.50	1889.4
	5	0.62	503.1
	6	0.70	961.4
	7	0.76	1524.7
	1	0.29	837.4
	2	0.34	1868.5
	3	0.41	600.0
15 μL	4	0.50	2505.9
	5	0.62	638.1
	6	0.70	1084.5
	7	0.74	1548.8
	8	0.80	877.2
	1	0.28	997.2
	2	0.33	2358.8
	3	0.40	689.0
20 μL	4	0.49	3439.8
	5	0.61	1103.6
	6	0.69	933.0
	7	0.74	2222.8
	8	0.80	1062.5

Table 3

HPTLC profile of the SF.

Volume of SF applied	Peak	R_f	Height area
	1	0.16	195.5
	2	0.19	262.6
	3	0.23	149.0
	4	0.29	2549.7
5 μL	5	0.34	6260.4
	6	0.41	1743.5
	7	0.50	10379.4
	8	0.61	1863.2
	9	0.69	596.7
	10	0.76	1342.3
	1	0.12	109.1
	2	0.16	424.0
	3	0.20	1312.0
	4	0.28	4143.7
	5	0.23	299.3
10 μL	6	0.33	9381.1
	7	0.41	3205.5
	8	0.50	16303.8
	9	0.61	3360.9
	10	0.69	823.9
	11	0.75	796.6
	1	0.16	379.9
	2	0.20	1574.0
	3	0.28	5098.2
	4	0.33	10890.1
	5	0.40	4536.3
15 μL	6	0.49	20910.9
	7	0.60	4063.2
	8	0.69	848.7
	9	0.74	958.3
	1	0.20	1950.2
	2	0.28	6013.3
	3	0.33	11055.3
	4	0.40	9566.2
20 μL	5	0.49	17840.5
	6	0.60	4102.5
	7	0.69	769.3
	8	0.75	1373.3

HPTLC profile of AESF and SF under UV 366 and 254 nm is recorded in the Figures 1 and 2, respectively.

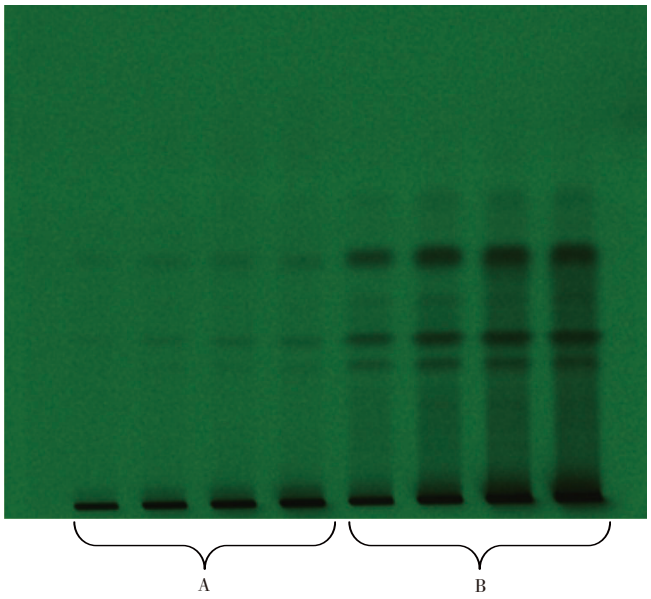

Figure 1. HPTLC picture of the AESF and SF of sample application at UV 254 nm.
A: AESF at 5, 10, 15 and 20 μL; B: SF at 5, 10, 15 and 20 μL.

The corresponding HPTLC chromatograms are presented in Figures 3A–3H.

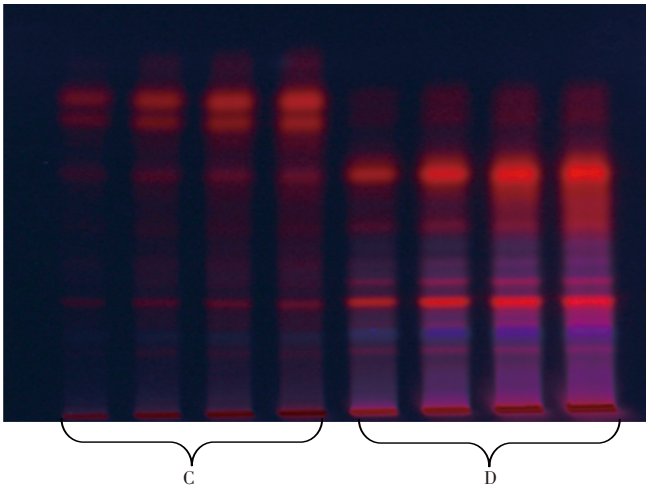

Figure 2. HPTLC picture of the AESF and SF of sample application at 366 nm.
C: AESF at 5, 10, 15 and 20 μL; D: SF at 5, 10, 15 and 20 μL.

The AESF revealed 6, 7, 8 and 8 spots with R_f values in the range of 0.28 to 0.80 for 5, 10, 15 and 20 μL application volume, respectively (Table 2 and Figures 3A–3D). However,

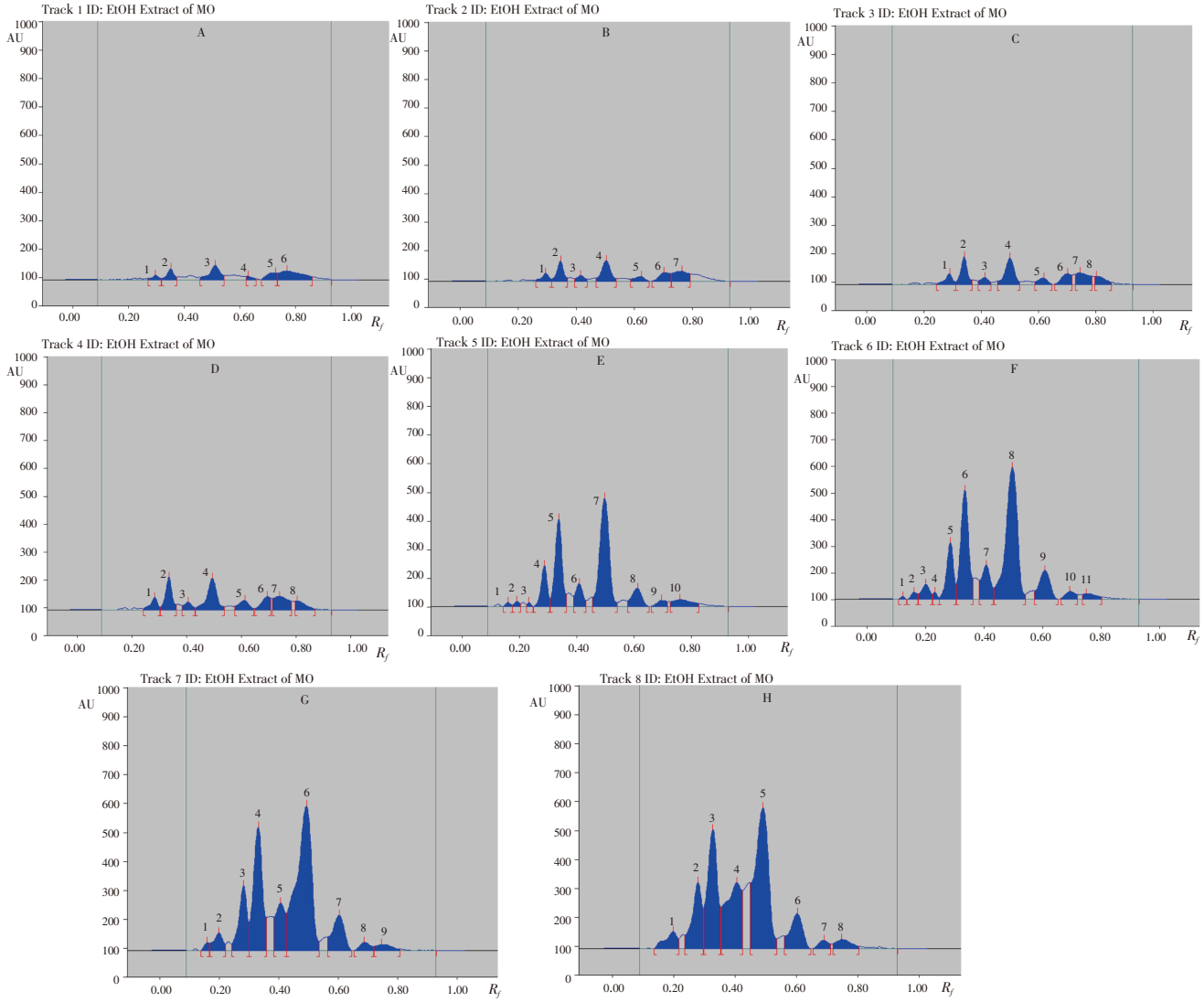

Figure 3. HPTLC chromatogram of AESF and SF showing different peaks of phytoconstituents.
A: AESF (5 μL); B: AESF (10 μL); C: AESF (15 μL); D: AESF (20 μL); E: SF (5 μL); F: SF (10 μL); G: SF (15 μL); H: SF (20 μL). MO: *M. obscura*.

SF revealed 10, 11, 9 and 8 spots with R_f values in the range of 0.12 to 0.76 for 5, 10, 15 and 20 μL application volume, respectively (Table 3 and Figures 3E-3H). The purity of the sample was confirmed by comparing the absorption spectra at start, middle and end position of the band.

3.3. Anti-inflammatory activity of AESF and SF

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 AESF and SF of *M. obscura* in doses of 100 and 200 mg/kg inhibited formalin-induced inflammation by 50% and 45.5%, 55.9% and 51.4%, respectively (Table 4).

Table 4
Effect of AESF and SF on formalin-induced inflammation in rats.

Treatment	Dose (mg/kg)	Paw edema volume (mL)	Inhibition (%)
Control	0.1 mL (formalin)	0.68±0.06	0.0
AESF	100	0.34±0.04*	50.0
	200	0.30±0.01**	55.9
SF	100	0.37±0.02*	45.5
	200	0.33±0.01**	51.4
Indomethacin	30	0.10±0.02**	85.2

4. Discussion

The dose-dependent anti-inflammatory activity of both extracts may explain at least partly the rationale beyond using the *Maytenus* species in treatment of inflammatory diseases[1]. Phytochmical screening of both extracts revealed the presence of flavonoids which are known to possess anti-inflammatory and antioxidant activities[22]. Flavonoid content of both extracts are almost equal and hence both extracts have close percentage of inhibition on inflammation. However, it is evident that they are different in polarity and UV absorption. Therefore, the presence of these compounds might be the ultimate cause for their bioactivity. HPTLC analysis of AESF and SF extracts revealed different peaks which are distinct for each extract and are concentration-dependent. Therefore, any additive or coamixed material will definitely affect the HPTLC picture showing new unrelated peaks pointing to the presence of either adulteration or deterioration. 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[13].

Hence, HPTLC provides great utility for the quality control of these products. It may be utilized to estimate the extent of activity according to the percentage content of these UV active compounds to the present extract's content. This might form a target for more comprehensive studies. The presence of any adulterant will be easily traced having these profiles developed and stored in a library. Literature review revealed that there is no previous work that has been done on these two extracts. AESF and SF of *M. obscura* produced dose-dependent significant anti-inflammatory activity in rats. This activity may be partially explained in view of the high content of flavonoids in both extracts. HPTLC profiles of both extrats revealed the presence of eight to nine major spots absorbing in different UV wavelengths, pointing to the difference in these compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Many countries (including developed ones) suffer a big problem in standardizing and quality control of the herbal plants. This is due to many factors among which are the complex form of these products and the inability of the traditional methods to precisely estimate the quality of the herbs. Regulatory authorities used to follow tedious procedures that may give indication about the quality of the herb but not complete map of the chemical content (which is vital for the biological activity of these plants).

Research frontiers

The research represents an advancement in the quality control of the herbal medicine. It provides new tool for fingerprinting using precise chemical contents mapping which might be utilized in correct identification of not only identity of the plant but even the purity of the plant product and the absence of contaminants. The authors utilized one of the advanced technologies *i.e.* HPTLC for that purposes which affords fast, reliable and precise tool for this purpose.

Related reports

No similar reports were found in the literature regarding this plant specifically, since it is being evaluated using this tool for the first time as per literature review available

in our resources.

Innovations and breakthroughs

The previous pharmacopeial methods for testing quality control concentrate on organoleptics, ash, and vague chemical screening which could not give precise chemical contents. Chemical content is the driving force for the medicinal value of the plant. This is why using such new techniques assure not only identity, purity of the plant but even the activity against the claimed diseases.

Applications

It can help to sensitize other groups of researches and adapt similar methodology in quality studies of other plants. It can be utilized even for microbial fermentation products and the presence of the analyte of interest. It may play a good role in food, food supplement screening and quality control.

Peer review

The research represents advanced methodological qualitative analysis of complex matrix such as herbal medicine where only very limited information can be acquitted using traditional techniques. The manuscript is well written. It has been organized according to well documented researches. The materials and methods are written precisely and well referenced. The results are compatible with what the methods may generate. The results were discussed scientifically according to the results obtained.

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