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HPTLC profile of aqueous extract of different chromatographic fractions of *Aloe barbadensis* Miller

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ABSTRACT

Objective: To establish the fingerprint profile of *Aloe barbadensis* Miller by using High Performance Thin Layer Chromatography (HPTLC) technique. **Methods:** after optimization of solvent system by Thin Layer Chromatography, different fractions of aqueous extract of *Aloe* was collected by column chromatography and the fingerprinting was done by High Performance Thin Layer Chromatography (HPTLC). **Results:** solvent system of methanol:chloroform :: 1:1 was found appropriate for fractionation by column chromatography. Total seven fractions were collected and they were analysed by HPTLC. Distinct spectra of different constituents were obtained for each fraction. **Conclusions:** HPTLC fingerprinting of *Aloe barbadensis* Miller may be useful in characterization of different phytochemicals found in this species. It may also be useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry.

1. Introduction

Aloe is a genus containing about 500 species of flowering succulent plants. The genus is native to Africa, and is common in South Africa's Cape Province, the mountains of tropical Africa, and neighboring areas such as Madagascar, the Arabian peninsula, and the islands of Africa [1]. The most common and well known species of genus *Aloe* is *Aloe vera*, or "true *aloe*". The species does not have any naturally occurring populations, although closely related *aloes* do occur in northern Africa. The APGIII system (2009) places the genus in the family Xanthorrhoeaceae, subfamily Asphodeloideae [2].

In the field of alternative medicine, *Aloe* spp. had been used for the treatment of many ailments by folks of many countries since long. Historical use of various *Aloe* species by humans is well documented [3]. Of the 500+ species of *Aloe*, only a few were used traditionally as a herbal medicine, *Aloe vera* (*Aloe barbadensis* Miller) again being the most commonly used species of *aloe* in herbal medicine. Also included are *Aloe perryi* (found in northeastern Africa) and *Aloe ferox* (found in South Africa) [4]. Documentation of

the clinical effectiveness is available, although relatively limited [5]. Extracts from *A. vera* are widely used in the cosmetics and alternative medicine industries, being marketed as variously having rejuvenating, healing or soothing properties [6]. There is, however, little scientific evidence of the effectiveness or safety of *A. vera* extracts for either cosmetic or medicinal purposes [7], and what positive evidence is available is frequently contradicted by other studies [6].

There are more than 200 compounds from *Aloe* spp. have been characterized, about 75 of which have biological activity [8]. *Aloe* spp. leaves contain several phytochemicals which may have possible bioactivity, such as polysaccharides, acetylated mannans, polymannans, anthraquinone, C-glycosides, flavonoides, tannins, saponins, phenols, anthrones and anthraquinones, and various lectins [9].

Many chemical compounds have been identified and separated from different *Aloe* sp. with the help of several biophysical techniques like chromatography, mass spectroscopy and nuclear magnetic resonance [10–13]. Reports about the phytochemical composition of the most common *aloe* species i.e. *Aloe vera* (*Aloe barbadensis* Miller) are very few and this fact prompted us to carry out this study which aims to do the fractionation of different chemical entities from the crude plant extracts. Thin Layer

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Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) are basic separation techniques commonly used in analytical chemistry for fractionation of different chemical compounds from the crude plant extracts. In the present study, we aim to get different fractions of aqueous extract from green variety of *Aloe vera* leaf powder by using column chromatography and densitometric analysis of those fractions will be done by HPTLC in order to get the preliminary idea about the phytochemical constituents of those fractions.

2. Materials and methods

2.1 Plant material

The germplasm of *Aloe vera* (green variety) were obtained from Defense Research Laboratory, Pithoragarh (Uttarakhand, India)

2.2 Preparation of aqueous extracts from *Aloe spp.*

Water is a polar solvent. The hot water extract contains water soluble fraction of *Aloe vera*. The *Aloe vera* powder (20g) was weighed, to this 100 ml of hot water (600 °C) was added, sealed the opening with parafilm and aluminum foils and was mixed gently. The solution was kept in an incubator shaker at 37 °C with gentle swirling at 120 rpm for overnight. The solution was filtered with a muslin cloth and doubly filtered with Whatman filter paper No.1. Then filtrate was centrifuged at 5000 rpm at 37 °C for 10 min. The supernatant was collected and spread on a large glass plate inside fan equipped incubator at 50 °C to make it dry. After 24 hour, semi-dried gel was collected and lyophilized in vacuum lyophilizer. The yield per gram of sample was calculated.

2.3 Thin Layer Chromatography:

TLC was done on the glass plates coated with Silica Gel-G. 10 µL of hot water extract was loaded on TLC plate and chromatography was performed by taking different solvents as the mobile phase. Plates were dried and spots were visualized by putting the plates in Iodine chamber. We observed that a combination of methanol and chloroform gave maximum separation of *Aloe* constituents. A gradient system of solvents was used (methanol: chloroform 3:7 to 7:3). Different no. of spots were observed for each solvent combination. Since results showed that maximum no. of spots were observed in methanol:chloroform(1:1), hence it was most suited for the separation and fractionation of *Aloe vera* compounds.

2.4 Column chromatography

optimized gradient of solvent combination [methanol:chloroform (1:1)] was used as mobile phase in silica gel adsorption column chromatography. One gram

of the hot water extract of *Aloe vera* was loaded in the glass column packed with silica gel (mesh size 60–120) and 200 ml of solvent system was used for running the column. Fractions (20 ml.) were collected at 15 min. interval.

2.5 HPTLC–photodensitometry

It is mandatory to investigate the quality of *Aloe spp.* extracts by densitometric analysis in order to know the chemical nature of those fractions. To study the possible composition of *Aloe vera* extract, TLC plate (20 × 10 cm), precoated with silica gel 60 F254 (E.Merck) (0.2mm thickness) with aluminum sheet support were used. The spotting device was a CAMAG Linomat V Automatic Sample Spotter (CamagMuttentz, Switzerland); the syringe, 100 µL (fromHamilton); the developing chamber was a CAMAG glass twin trough chamber (20 × 10 cm); the densitometer consisted of a CAMAG TLC scanner 3 linked to WINCATS software. Saturation time for mobile phase was 2 hours. 10 µL each of the fractions were spotted (band width: 6 mm) on HPTLC plates. Chromatograms were developed for 9.0 cm using [methanol:chloroform (1:1)] as a mobile phase. Development of the plates was done by spraying with anisaldehyde sulphuric acid reagent and the spots were detected by heating the plate at 105–110 °C for 5 min. The developed plates were then scanned to record the peak areas. A plot was drawn by keeping Rf values on X-axis and absorbance on Y-axis.

3. Results

Hot water extract of *Aloe vera* was lyophilized in vacuum lyophilizer at –55 °C. The yield (w/w) was 0.275 g. Without the knowledge of actual chemical nature, it is very difficult to ascertain the most suitable solvent for purification of the compounds. So in order to find out the most suitable solvent system (in which maximum no. of constituents can be separated), we used different organic solvents like carbon tetrachloride, acetone, ether, methanol, chloroform and combinations of them in Thin Layer Chromatography. We observed that a combination of methanol and chloroform gave maximum separation of *Aloe vera* constituents. A gradient system of solvents was used (methanol: chloroform 3:7 to 7:3). Different no. of spots were observed for each solvent combination (Fig.1). Maximum no. of spots were observed in methanol:chloroform (1:1) solvent system, hence it is most suited for the separation and fractionation of *Aloe vera* compounds (Table 1). This particular combination of solvent was used as mobile phase in silica gel adsorption column chromatography and HPTLC. Total seven fractions were obtained through column chromatography. Each fraction contained different chemical compounds having different polarity. Less polar compounds were separated out first followed by more polar compounds. Further characterization of those separated compounds in different

fractions was done with HPTLC (Fig.2). Spectral analysis of all seven fractions showed multiple peaks in the range of 200 nm to 410 nm (Fig.3). Most substances have characteristic spectra and can be identified tentatively based on this property.

Table 1

separation of compounds of hot water extract of *Aloe vera* on TLC using the gradient of combination of two solvents methanol and chloroform.

Sl. No.	Solvent system methanol and chloroform	No.of spots	Rf values of spots
1.	3:7	2	0.38, 0.76
2.	2:3	3	0.11, 0.76, 0.88
3.	1:1	4	0.42, 0.62, 0.76, 0.89
4.	3:2	3	0.09, 0.68, 0.78
5.	7:3	1	0.74

Solvent system of methanol and chloroform in the ratio of 1:1 is most suitable for separation

Table 2

HPTLC profile of fractions of aqueous extract of *Aloe vera*

Fraction	Assigned substance (no.)	Rate of flow (Rf)	Absorbance maxima
1	2	0.24	200AU, 375nm
	5	0.37	138AU, 306nm
	6	0.60	132AU, 294nm
	3	0.63	130AU, 294nm
	4	0.73	160AU, 288nm
2	1	0.89	218AU, 220nm
	7	0.21	80 AU, 306 nm
	2	0.24	88 AU, 306 nm
	3	0.28	75 AU, 340 nm
	6	0.61	100AU, 371nm
3	1	0.91	240AU, 221nm
	2	0.24	82 AU, 365 nm
	5	0.35	22 AU, 382 nm
4	1	0.93	162AU, 200nm
	3	0.24	72 AU, 370 nm
	7	0.23	75 AU, 305 nm
	2	0.25	71 AU, 376 nm
	4	0.43	22 AU, 410 nm
5	6	0.65	48 AU, 375 nm
	1	0.89	150AU, 219nm
	1	0.26	100AU, 311nm
	2	0.51	60 AU, 308 nm
	3	0.68	75 AU, 341 nm
6	4	0.90	90 AU, 200 nm
	1	0.20	82 AU, 311 nm
	2	0.26	110AU, 312nm
	3	0.31	125AU, 376nm
	4	0.45	50 AU, 289 nm
7	5	0.91	125AU, 200nm
	1	0.22	162AU, 311nm
	2	0.32	248AU, 310nm
	3	0.48	105AU, 334nm
	4	0.65	75AU, 303 nm
	5	0.78	58AU, 200 nm
6	0.87	55AU, 260 nm	

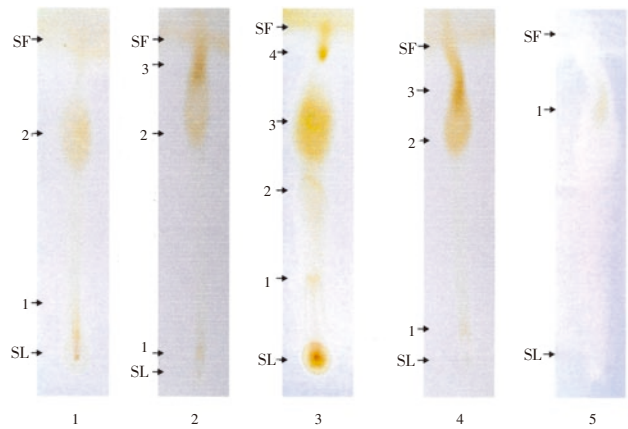


Figure 1. Thin Layer Chromatography of hot water extract of *Aloe vera* using the gradient of combination of two solvents methanol and chloroform(3:7, 2:3, 1:1, 3:2, 7:3)

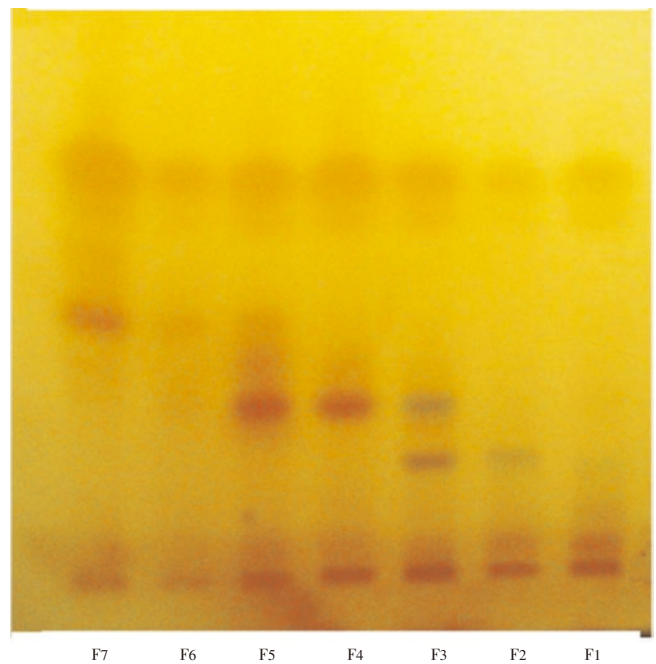
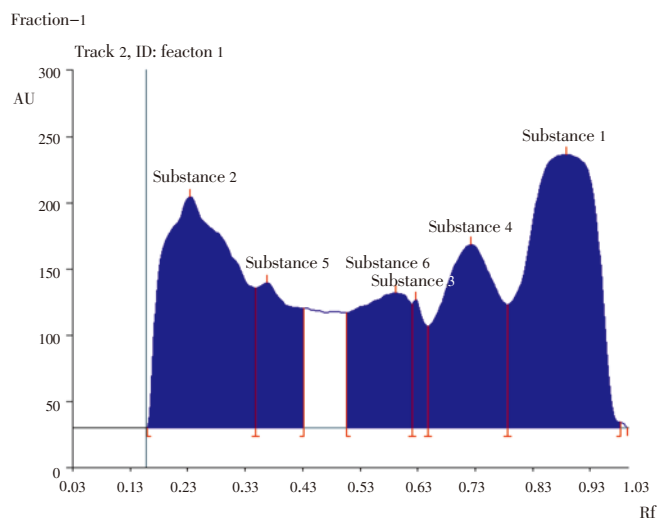


Figure 2. HPTLC profile of fractions of hot water extract of *Aloe vera* (F1–F7 all seven fractions)



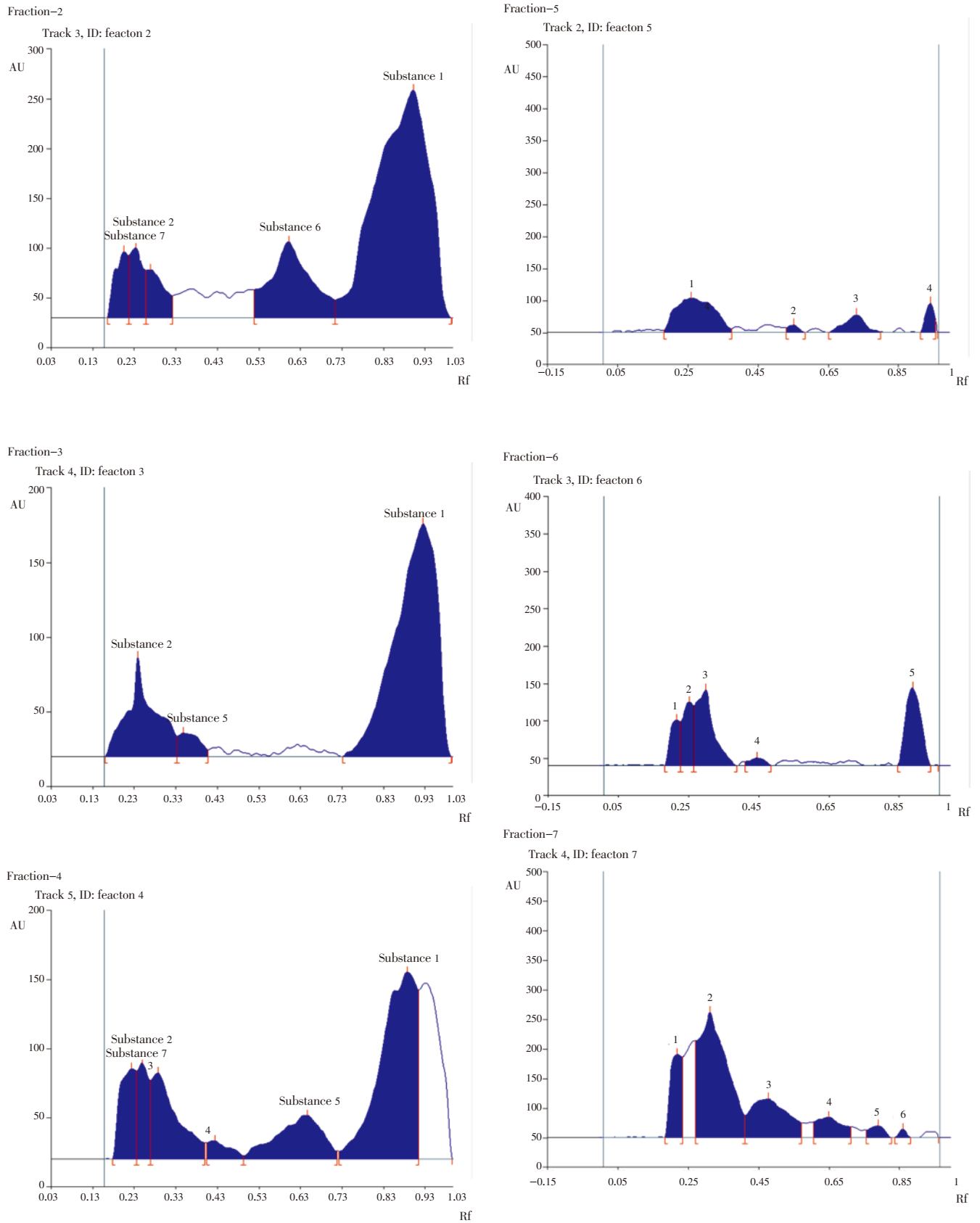


Figure 3. HPTLC chromatogram of different fractions showing different peaks of phytoconstituents.

4. Discussion

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants.

Aloe sp. have been used for several purposes since ancient times. Different preparations made by different parts of the plant has been used in different ailments but their exact chemical components were unknown for a long time. Several attempts have been made previously to characterize different phytosterols of *Aloe* sp. Some compounds have been purified and their pharmacodynamics have been studied in animal models. Present study is the first report pertaining to deduce the varied composition of *Aloe* extract using the HPTLC technique. In this study we observed different absorbance of different fractions of *Aloe* extract. The absorbance maxima at the wavelengths of 260 nm is characteristic of carbohydrates and galactans. Other peaks at 230–290 nm correspond to glycoproteins and different protein constituents. Multiple peaks correspond to various pigments and the other lipid soluble compounds like alkaloids, flavonoides, steroids, anthraquinones etc.

Several peaks observed in this experiment indicate the diverse composition of aqueous extract of *Aloe vera*. The data and HPTLC fingerprint profile of aqueous extract of *Aloe vera* leaves could be used as a valuable analytical tool in the routine quality control and standardization of *A. vera* gel and products made from them. The fingerprint profile will also be useful in differentiating *Aloe barbadensis* Miller with other species of *Aloe* genus. Further characterization of these fractions by applying more sophisticated separation and purification techniques are necessary to find out the exact chemical compounds and their relative abundance. Some of which might possess the therapeutic property. Those molecules may have commercial significance in future.

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Conflict of interest statement

not declared

References

- [1] Akinyele B.O., Odiyi A.C. Comparative study of the vegetative morphology and the existing taxonomic status of *Aloe vera* L. *Journal of Plant Sciences* 2007; **2**(5): 558–63.
- [2] Stevens, P.F. (2001) onwards. Angiosperm Phylogeny Website: Asparagales: Asphodeloideae
- [3] Haller J.S. Jr. A drug for all seasons medical and pharmacological history of *Aloe*. *Bull. N.Y. Acad. Med.* 1990; **66**(6): 647–59.
- [4] Shelton R.M. *Aloe vera*: its chemical and therapeutic properties. *Int J. Dermatol* 1991; **30** (10): 679–83.
- [5] Vogler B.K., Ernst E. *Aloe vera*: a systematic review of its clinical effectiveness. *Br J Gen Pract* 1999; **49**(447): 823–28.
- [6] Eshun K, He Q. *Aloe vera*: a valuable ingredient for the food, pharmaceutical and cosmetic industries—a review. *Crit Rev Food Sci Nutr.* 2004; **44**(2): 91–6.
- [7] Marshall J.M. *Aloe vera* gel: What is the evidence? *The Pharmaceutical Journal* 1990; **24**: 360–62.
- [8] Surjushe A., Vasani R., Saple D.G. *ALOE VERA*: A SHORT REVIEW *Indian J. Dermatol.* 2008; **53**(4): 163–66.
- [9] Wintola O.A., Afolayan A.J. Phytochemical constituents and antioxidant activities of the whole leaf extract of *Aloe ferox* Mill. *Pharmacogn Mag.* 2011; **7**(28): 325–33.
- [10] Ranghoo–Sanmukhiya M, Govinden–Soulange J, Laverigne C, Khoyratty S, Da Silva D, Frederich M, Kodja H. Molecular biology, phytochemistry and bioactivity of three endemic *Aloe* species from Mauritius and Réunion Islands. *Phytochem Anal.* 2010; **21**(6): 566–74.
- [11] Fanali S, Aturki Z, D’Orazio G, Rocco A, Ferranti A, Mercolini L, Raggi M.A. Analysis of *Aloe*–based phytotherapeutic products by using nano–LC–MS. *J Sep Sci.* 2010; **33**(17–18): 2663–70.
- [12] Abd–Alla H.I., Shaaban M., Shaaban K.A., Abu–Gabal N.S., Shalaby N.M., Laatsch H. New bioactive compounds from *Aloe hijazensis*. *Nat Prod Res.* 2009; **23**(11): 1035–49.
- [13] Karagianis G, Viljoen A, Waterman PG. Identification of major metabolites in *Aloe littoralis* by high–performance liquid chromatography–nuclear magnetic resonance spectroscopy. *Phytochem Anal.* 2003; **14**(5): 275–80.