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Comparative Analysis of Antimicrobial Activity of Methanolic Extracts of *Aloe Vera* and Quantification of Aloe-Emodin Collected From Different Climatic Zones of India

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Abstract

Background: *Aloe vera* is a plant of Liliaceae family has been traditionally used for a variety of medicinal purposes. It has multiple applications such as emollient, purgative, antibacterial, antioxidant, antifungal, antiseptic and in cosmetics industries. The aim of present study was standardization of HPLC protocol for quantification of aloe- emodin and study of antimicrobial activity of methanolic extracts of *Aloe vera* collected from different climatic regions of India against pathogenic reference ATCC strains.

Methods and findings: Antibacterial and antifungal activity was checked by agar well diffusion method. High Performance Liquid Chromatography was used for quantification of aloe- emodin from collected samples by comparing with standard aloe-emodin marker compound. The different accession of *Aloe vera* methanolic extracts revealed significant antimicrobial activity against the tested strains. Extracts were more active against gram negative bacteria than gram positive ones. Amounts of marker compound aloe- emodin concentration varied a lot in different accessions. Climatic conditions showed notable effect on quantity of marker compound.

Conclusion: The present study has showed the role of climatic conditions and geographical locations on the variation in amounts of aloe- emodin present in different crude extracts of Aloe vera due to presence of which it has showed strong antimicrobial activity.

Keywords: *Aloe vera*; Anthraquinone; Aloe- emodin; Antimicrobial activity; MIC; Phytochemicals

Introduction

Aloe vera is a shrubby, perennial succulent plant of Liliaceae family having turgid pea-green leaves joined at the stem in a rosette pattern. *Aloe vera* plant is characterized by stemless large, thick, fleshy leaves having a sharp apex and a spiny margin [1]. Aloe vera has been traditionally used for a variety of medicinal purposes. It is an inseparable part of indigenous medicine system of India. It has gained high importance for its diverse therapeutic properties. Aloe vera contains over 75 nutrients and 200 active compounds; including vitamins, enzymes, minerals, sugars, lignin, anthraquinones, saponins, salicylic acid and amino acids which are responsible for their medicinal properties [2]. Its secondary metabolites have multiple applications such as emollient, purgative, antibacterial, antioxidant, antifungal, antiseptic and in cosmetics industries [3-6]. Aloe vera gel has been used since early times for the topical treatment of various skin conditions such as cuts, burns and eczema [7]. *Aloe vera* has been shown to have anti inflammatory activity [8,9], immuno stimulatory activity [10] and cell growth stimulatory activity [11]. Furthermore, activity

against a variety of infectious agents has been attributed to *Aloe vera*; for instance antiviral [12] and antifungal [13].

Aloe vera contains free anthraquinones and their derivatives like Barbaloin, Aloe-emodin-9-anthrone, Isobarbaloin, Anthrone-C-glycosides and chromones. In large amounts these compounds exert a powerful purgative effect while in smaller quantity; they appear to aid absorption from the gut therefore it acts as potent antimicrobial agents [14]. Specific plant compounds such as anthraquinones [15,16] and dihydroxy- anthraquinones [17], as well as saponins [18] have been proposed to have direct antimicrobial activity.

Aloe- emodin (1,8-dihydroxy-3-(hydroxymethyl)anthracene-9,10-dione) derived from leaves of *Aloe vera* is an antimicrobial compound [19, 20]. It is also known to have anticancerous activity in neuroectodermal tumors [21], lung squamous cell carcinoma [22], hepatoma cells [23], in a glia cell line [24] and a human glioma cell line [25,26]. Present study focuses on antimicrobial activity of methanolic extracts of *Aloe vera* from 12 different accessions covering 6 agro- climatic regions of India. It also deals with standardization of quantities of antimicrobial compound aloe- emodin from different accession.

Experimental Section

Collection of plant

Samples were collected from 12 sites covering 6 agro-climatic zones of India. Each site had 2 sub-sites. Samples were collected in the months of Jan-Feb 2013. Healthy leaves of *Aloe vera* were collected from individual plants at each location. Different collection sites have been depicted in **Figure 1.** Geographical locations and average rainfall of these sites have been given in **Table 1.** The plant material was identified and authenticated by comparing the herbarium specimen available in Department of Genetics, M. D. University, Rohtak (India). Herbarium specimen number is MDU-6803. Tissues were placed in sterile plastic bags and brought in an ice box, sealed properly. All samples were brought to the laboratory and processed further.

Preparation of Crude Plant Materials

The collected leaves were chopped into small pieces and shade dried at room temperature. 100 gm dried leaves pieces were grinded and soaked in 60% ethanol solution for 24 hrs. Sulphuric acid and chloroform were added into the extractive and refluxed to remove the chloroform extractive [27]. The extract was prepared by cold percolation method. Stock solution was prepared by dissolving in methanol with the help of rotary shaker. A reddish brown colloid containing aloe extractive was obtained, which is filtered by using Whatman filter paper. After the evaporation of the methanol, a yellowish-brown colloid was obtained as crude extract.

Antimicrobial Evaluation

Reference Strains: Pathogenic reference ATCC (American Type Culture Collection) strains were used for checking activity. 9 bacterial and 2 fungal strains were used for the study. Strains were procured from Post Graduate Institute of Medical Sciences, Rohtak (Haryana). India. Out of 9 bacterial strains, 7 were gram negative viz. *Shigella flexneri* ATCC 12022, *Proteus mirabilis* ATCC 43071, *Salmonella typhi* ATCC 13311, *Serratia marcescens* ATCC 27137, *Klebsiella pneumonia* ATCC 700603, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and 2 were gram positive viz. *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 259323. Fungal strains were *Candida albicans* ATCC 3018 and *Aspergillus niger* ATCC 282).

In-vitro Antibacterial and Antifungal assay

Antibacterial and antifungal activity was checked by agar well diffusion method. 100 mg of the methanolic extract was dissolved in 1 ml of DMSO and used as test sample. Fresh overnight grown culture was used for inoculums preparation. Inoculums were prepared in peptone water and incubated for 2 hours. Turbidity was adjusted equivalent to 0.5 McFarland units (approximately 10^8 CFU/ml). Inoculums were spread over fresh nutrient agar plates with a sterile spreader. 4 wells of 6 mm diameter were cut with the help of a sterile cutter. Control disc was placed in centre. 10, 20, 30 and 40 µl of this prepared test sample was added to all bacterial strains used and DMSO was used as negative control. 10 µg streptomycin and 10 µg ketoconazole discs were used as positive control respectively for antibacterial and antifungal activity. Both were purchased from Himedia laboratories Pvt. Ltd (India). Plates were incubated for 24 hours at 37°C. Clear zone of inhibition around each well was measured with help of standard ruler HiAntibiotic ZoneScale™-C supplied by HiMedia Laboratories Pvt. Ltd. India. Each experiment was done in triplicates.

Minimum Inhibitory Concentration [MIC]

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The MIC

Figure 1 Different collection sites of *Aloe vera* from different places of India

values of extracts were determined based on a micro broth dilution method in 96 multi-well micro titer plates developed by Sarkar et al. [28] with slight modifications. 50 µl of nutrient broth and 50 µl of normal saline were added to each well of plate. 10 µl of resazurin indicator solution (prepared by dissolving a 270 mg tablet in 40 ml of sterile distilled water) was added in each well. A volume of 100 µl of test materials (stock concentration 10 mg/ml of extracts) was added into the first row of the plate. Serial dilutions were performed such that each well had a total of 100 µl of the test material in serially descending concentrations. Finally 10 µl of bacterial suspension concentration of 5 x 10^6 CFU/ml was added to each well. Plate had a column with streptomycin as positive control. The plates were prepared in triplicate and placed in an incubator at 37°C for 18 to 24 hours. Any colour change from purple to pink indicates growth of microbes. The highest dilution at which no color change occurred was taken as the MIC value of extract and was expressed in mg/ml.

HPLC Analysis

Chemicals used

Aloe-emodin (used for the control of retention times), HPLC grade methanol, acetonitrile and phosphoric acid were purchased from Sigma-Aldrich (USA). AR grade ethanol, sulfuric acid and chloroform were used for the extract preparation. Ultra pure water (18.2 MΩ·cm−1) was obtained by means of a Milli Q apparatus by Millipore, USA. Stock solutions of the analyte (1 mg/mL) were prepared by dissolving pure substance in methanol and were stable for at least two months when stored at −20°C (as assessed by HPLC assays). Standard solutions were prepared daily by diluting stock solutions with the mobile phase and directly injected into the HPLC system.

Instrumentation

A High Performance Liquid Chromatography equipped with gradient elution capability, Ultraviolet spectrophotometer (UV) and photodiode array (PDA) as detector and an auto sampler (Agilent Technologies system, model 1260 Infinity, USA). A stainless steel C18 column, length of 250 mm, internal diameter 4.6 mm and 5 µm particle size (250 mm × 4.6 mm 5 µm) column was used. The column temperature maintained at ambient condition.

HPLC Analysis Condition

The mobile phase consisted of acetonitrile (A) and 0.1% aqueous phosphoric acid (B) with a gradient elution of 24% A at 0-12 min, 24-50% A at 12-22 min, 50-24% A at 22-40 min and 24% A at 40-50 min. The flow rate was 1.0 ml/min and the separation was monitored by absorbance at 254 nm. The injection volume was 20 µl. The identification of peaks was done by comparing HPLC chromatograms of individuals with the peak of purchased standard aloe- emodin.

Results

Methanolic leaf extracts of *Aloe vera* were prepared. Yield of extracts have been shown in **Table 2**. Maximum yield was obtained from Punjab (4.3 gm) and Haryana (4.2 gm) accessions. Minimum yield was from Kerala (3.2 gm). All extracts showed the activity against all reference bacterial and fungal strains. Antimicrobial activities were ranged from 11.50 mm to 20.50 mm as measured by diameter of zone of inhibition. Result of zone of inhibition of all accessions and control against test strains have been given in **Table 3**. Antibacterial activity for some of the A. vera accessions have been given in **Figure 2**.

Maximum antibacterial activity was noted against E. coli with a zone of inhibition of 20.50 mm and minimum activity was against P. aeruginosa with 11.83 mm zone of inhibition (**Table 3**, **Figure 2**). Activity against Shigella flexneri Klebsiella pneumoniae and E. coli was quiet good as compared to control. Extracts were more active against fungus C. albicans than A. niger. Punjab, Haryana and West Bengal accessions showed good activity against most of the tested strains. Significant activity was reported against K. pneumonia with a maximum 16 mm zone of inhibition by West Bengal accession. Control showed only 12 mm zone of inhibition against this bacteria. Minimum activity was shown against P.

Table 1 Geographical locations and average rainfall of plant collection sites

S.No.	Accessions names	Place of collection	Latitude	Longitude	Average rainfall (mm)
1	Jammu	Jammu	34° 44' N	78° 54' E	1,011
2	Himachal Pradesh	Palampur	32° 29' N	78° 10' E	1,251
3	Punjab	Sangrur	30° 40' N	75° 50' E	649
4	Haryana	Rohtak	30° 30' N	76° 60' E	617
5	Rajasthan	Jaisalmair	27° 00' N	74° 00' E	209.5
6	Gujarat	Gandhinagar	23°.00' N	72°.00' E	1,107
7	Uttar Pradesh	Pratapgarh	27° 40' N	80° 00' E	904
8	Madhya Pradesh	Bhopal	23° 30' N	80° 00' E	1,146
9	West Bengal	Kolkata	23° 00' N	87° 00' E	1,582
10	Andhra Pradesh	Hyderabad	16° 00' N	80° 00' E	812.5
11	Goa	Kochi	15° 00' N	74° 25' E	3,055
12	Kerala	Vasco	10° 00' N	74° 06' E	3,005

Table 2 Yield of *Aloe vera* extracts collected from different Agro- climatic zones

Agro-climatic zone	Name of State	Yield (g)
Highland	Jammu	4
	Himachal Pradesh (HP)	3.8
Semi-arid	Haryana	4.2
	Punjab	4.3
Arid	Rajasthan	3.6
	Gujarat	3.5
Humid Subtropical	Uttar Pradesh (UP)	4
	Madhya Pradesh (MP)	3.7
Tropical wet & dry	West Bengal (WB)	4.1
	Andhra Pradesh (AP)	3.9
Tropical wet	Goa	3.4
	Kerala	3.2

Table 3 Antimicrobial activity of different accessions of *Aloe vera* Crude extracts measured by zone of inhibition (mm ± SD)

Accessions	CA	AN	SF	EF	SA	PM	ST	SM	KP	EC	PA
Jammu	20.00 ± 10	17.83 ± 0.7	14.66 ± 0.5	14.00 ± 0.5	16.83 ± 0.7	14.66 ± 0.5	16.00 ± 0.5	14.16 ± 0.7	13.50 ± 0.5	15.16 ± 0.7	13.00 ± 1.0
HP	18.16 ± 07	18.0 ± 0.5	15.83 ± 0.7	14.66 ± 0.7	16.33 ± 0.5	14.83 ± 0.7	15.00 ± 0.5	12.50 ± 0.5	14.00 ± 0.5	16.00 ± 1.0	12.50 ± 0.5
Punjab	17.00 ± 05	16.16 ± 0.7	16.00 ± 1.0	14.33 ± 0.5	20.00 ± 0.5	13.33 ± 0.5	18.50 ± 0.5	13.00 ± 0.5	12.16 ± 0.2	19.00 ± 0.5	13.83 ± 0.7
Haryana	19.00 ± 0.5	15.66 ± 0.5	16.16 ± 0.7	13.00 ± 0.5	19.00 ± 1.0	16.50 ± 0.5	19.00 ± 0.5	14.16 ± 0.2	13.00 ± 0.5	20.50 ± 1.0	14.50 ± 0.5
Rajasthan	19.83 ± 0.7	17.33 ± 0.5	18.00 ± 1.0	16.00 ± 1.0	17.33 ± 0.5	15.5 ± 0.5	17.16 ± 0.2	13.83 ± 0.7	16.16 ± 0.2	18.50 ± 0.5	14.66 ± 0.5
Gujarat	17.16 ± 0.7	16.50 ± 0.5	14.33 ± 0.5	14.00 ± 1.0	17.00 ± 1.0	13.00 ± 0.5	18.5 ± 0.5	13.50 ± 0.5	13.33 ± 0.7	19.83 ± 0.7	13.66 ± 0.5
UP	19.50 ± 0.5	15.00 ± 1.0	14.66 ± 0.5	13.5 ± 0.5	20.16 ± 0.2	14.33 ± 0.5	17.33 ± 0.5	14.33 ± 0.5	13.50 ± 0.5	18.33 ± 0.5	14.00 ± 1.0
MP	18.00 ± 1.0	11.5 ± 0.5	16.00 ± 0.5	14.0 ± 0.5	18.5 ± 0.5	14.33 ± 0.5	15.83 ± 0.7	12.50 ± 0.5	14.50 ± 0.5	19.00 ± 1.0	12.50 ± 0.5
WB	17.66 ± 0.5	16.0 ± 1.0	16.00 ± 1.0	12.66 ± 0.5	19.16 ± 0.7	13.00 ± 0.5	18.16 ± 0.2	13.33 ± 0.7	16.00 ± 1.0	20.50 ± 0.5	13.50 ± 1.0
AP	19.00 ± 1.0	17.50 ± 0.5	16.83 ± 0.7	13.66 ± 0.5	20.83 ± 0.7	12.83 ± 0.7	17.33 ± 0.7	14.00 ± 0.5	14.83 ± 0.7	19.5 ± 1.0	14.00 ± 1.0
Goa	18.66 ± 0.5	16.66 ± 0.5	16.00 ± 0.5	12.00 ± 1.0	20.33 ± 0.5	13.00 ± 0.5	17.00 ± 0.5	13.50 ± 0.5	14.33 ± 0.7	19.00 ± 1.0	11.83 ± 0.7
Kerala	17.83 ± 0.7	15.00 ± 0.5	16.16 ± 0.7	13.83 ± 0.7	20.00 ± 1.0	14.83 ± 0.7	18.50 ± 0.5	14.50 ± 0.5	14.00 ± 1.0	19.00 ± 0.5	13.00 ± 1.0
Control	18.0 ± 1.0	23.0 ± 0.5	14.0 ± 1.0	23.0 ± 0.5	26.0 ± 1.0	23.5 ± 0.5	24.0 ± 0.5	25.5 ± 0.5	12.0 ± 1.0	13.0 ± 1.0	18.0 ± 1.0

Fungal strains = CA- *Candida albicans* and AN- *Aspergillus niger*
Bacterial strains = EC- *Escherichia coli*, PM- *Proteus mirabilis*, KP- *Klebsiella pneumoniae*, PA- *Pseudomonas aeruginosa*, SF- *Shigella flexneri*, SM- *Serratia marcescens*, ST- *Salmonella typhi*, EF- *Enterococcus faecalis* and SA- *Staphylococus aureus*
Control for fungal strains= Ketocanozole (10 µg)
Control for bacterial strains= Streptomycin (10 µg)

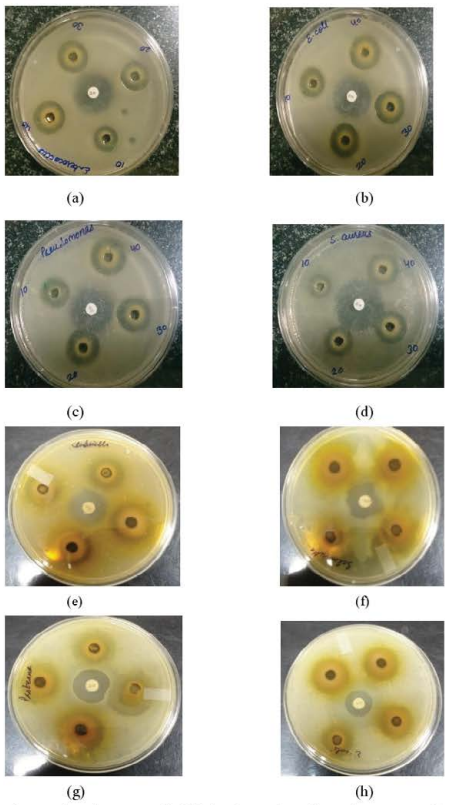

Figure 2 Plate showing zone of inhibition shown by methanolic crude extracts of *Aloe vera*; (a) Rajasthan accession against *Enterococcus faecalis*; (b) Haryana accession against *Escherichia coli*; (c) U.P accession against *Pseudomonas aeruginosa*; (d) A.P. accession against *Staphylococcus aureus*; (e) W.B. accession against *Klebsiella pneumonia*; (f) Punjab accession *Salmonella typhi*; (g) Rajasthan accession against *Proteus mirabilis*; (h) Jammu Accession against *Escherichia coli*

aeruginosa, S. marcescens, S. typhi, P. mirabilis and E. faecalis as compared with control. Jammu and HP accessions showed good antifungal activity. Extracts were more active against gram negative bacteria than gram positive ones. MIC values ranged from 1.2 mg/ml to 5 mg/ml. Minimum MIC value of 1.2 mg/ml was found against E. coli revealed by most of the accessions. Maximum MIC was against Proteus mirabilis, Pseudomonas aeruginosa and fungus A. niger shown by most of the accessions. MIC values of all the accessions have been shown in **Table 4.**

The HPLC analysis revealed that there was mark able difference in the quantity of aloe- emodin from different accessions that were collected throughout India on the basis of their different climatic zones. The results obtained indicated the presence of anthraquinone derivative aloe- emodin in each accession with the significant differences in their quantities. Important aspects like Sample name, Climatic zone, Retention time (RT), Area of peak and Yield percentages of compound (aloe- emodin) of HPLC analysis are given in the **Table 5**. HPLC chromatograms of standard along with 3 accessions viz Gujarat, Uttar Pradesh and Haryana have been shown in **Figures 3a-3d.**

RT's (retention time) of standard was 28.10 min. and all the desired peaks ranged from 28.06 to 28.91 min. Area of desired peaks ranged from 25.24 to 220.25 Mau*s (milli- absorbance unit). Maximum compound percentage was from the Punjab (Semi- arid zone) accession (0.16%) and minimum from the AP (Tropical wet and dry zone) accession (0.03%) as compared with standard (99%). The percentage of aloe-emodin in different accessions has been shown in **Figure 4.**

Discussion

The process of investigating plants to identify the chemical substances is of great interest to plant scientists because there is a need to discover new drugs for treating diseases. Secondary metabolites formed by living systems, notably from plant origin, have shown great potential in treating human diseases such as

cancer, coronary heart diseases, diabetes and infectious diseases [29]. These usually consist of the phenolic and polyphenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids and steroids distributed throughout the plant kingdom [30,31].

Aloe vera can grow in almost all types of environmental conditions but there are several factors that can influence the quality and quantity of a particular constituent. The type of solvents and methods of preparation affect antimicrobial activity of plants [32]. In present study, we prepared *A. vera* leaf gel extracts by using methanol as a solvent. Previous studies report that methanol as a solvent extracts revealed the antimicrobial properties of plants due to presence of phenolic compounds, saponin, bryophyllin and other secondary metabolites which are reported to be antimicrobial [33,34]. Methanolic extract induced the best extraction yield and more complex composition of phenolics [35].

Aloe vera plant is rich in alkaloids, tannins, saponins, flavonids, anthraquinones, barbaloins, glycosides and terpenoids [36]. So methanol is the appropriate solvent for preparation of extracts for antimicrobial study. Our results correlate with previous studies. Methanol extracts of all 12 accessions exhibited quite good antimicrobial activity. Previous studies on *Aloe vera* leaf gel extracts showed that extract was more effective against gram positive bacteria than gram negative [37,38]. In our study methanolic extract showed good activity against gram positive S. aureus and gram negative E. coli and S. typhi. Our extracts also showed good activity against both the fungal strains i.e. A. niger and C. albicans.

Antimicobial activity

Although all extracts were quite effective against all the tested strains. But there are variations in diameter of zones of inhibition. Solvent system and extraction procedure was uniform throughout the study. Variation in inhibition zones may be the due to differences in the phytochemical composition of different accessions. Accessions were from 6 climatic zones of India. Variations in the sensitivity of the bacterial and fungal species tested on the extracts might be because of differences in the local environmental factors. Previous studies states that mineral and phytochemical composition of plants is influenced by various environmental factors including the geography, climate, soil minerals, grazing stress, seasonal changes, phenological stages and ability of plant uptake of minerals from soil [39-41].

A good antimicrobial activity was also shown by Jammu and H.P. accessions. *Aloe vera* is a cold sensitive plant. During stress more phytochemicals are produced in plants to withstand the adverse conditions. Studies conducted on plants in stress conditions showed higher production of flavonoids, anthocyanins and mucilaginous substances in stress condition [42]. Flavonoids and anthocyanins are both antimicrobial in nature [43].

Quantification of Aloe- emodin

Standardization and characterization of herbal drugs is a topic of continuous scientific interest in the herbal drug industry. With the advent of modern chromatographic systems there is an ever increasing intent to produce and develop easy, rapid, convenient and cost effective methods for standardization [44]. Standardization of methanolic extract of plant leaves requires HPLC which is a sensitive and accurate tool widely used for the quality assessment of plant extract and its derived products and formulation [45]. The High Performance Liquid Chromatography represents the best technique used now days to separate, to fingerprint qualitatively and to identify or quantify each molecule from complex mixtures as is it the case of plant extracts and herbal supplements [46]. The use of multiple methods involving different mobile gradient phases would increase the validity and reliability of the obtained results.

There are not too much previous comparative studies on diversity bases which can correlates the marker compound analysis of *Aloe vera*. The results indicated that, *Aloe vera plant* collected from humid- subtropical and semi- arid zone showed better aloe- emodin production as compared to tropical area zone. Cassia, another plant genus which is a important source of

Table 4 Minimum Inhibitory Concentration (MIC in mg/ml) of various accessions of *Aloe vera* Extracts against different micro- organisms

Accessions	CA	AN	SF	EF	SA	PM	ST	SM	KP	EC	PA
Jammu	1.2	2.5	2.5	2.5	1.2	2.5	1.2	1.2	2.5	1.2	2.5
HP	1.2	2.5	2.5	2.5	1.2	2.5	1.2	1.2	2.5	1.2	2.5
Panjab	2.5	5.0	2.5	2.5	1.2	5.0	2.5	2.5	2.5	2.5	5.0
Haryana	2.5	5.0	2.5	2.5	1.2	5.0	2.5	1.2	2.5	1.2	5.0
Rajasthan	2.5	5.0	2.5	2.5	1.2	5.0	1.2	1.2	2.5	1.2	5.0
Gujarat	5.0	5.0	5.0	2.5	5.0	5.0	2.5	5.0	2.5	2.5	5.0
UP	1.2	5.0	5.0	2.5	1.2	5.0	2.5	1.2	2.5	1.2	5.0
MP	2.5	5.0	2.5	2.5	2.5	5.0	2.5	5.0	2.5	1.2	5.0
WB	5.0	5.0	2.5	2.5	1.2	5.0	1.2	2.5	2.5	1.2	5.0
AP	2.5	5.0	5.0	2.5	5.0	5.0.	2.5	5.0	2.5	2.5	5.0
Goa	5.0	5.0	5.0	2.5	1.2	5.0	1.2	5.0	2.5	1.2	5.0
Kerala	5.0	5.0	5.0	2.5	1.2	5.0	1.2	2.5	2.5	1.2	2.5

Fungal strains = CA- *Candida albicans* and AN- *Aspergillus niger*
Bacterial strains = EC- *Escherichia coli*, PM- *Proteus mirabilis*, KP- *Klebsiella pneumoniae*,
PA- *Pseudomonas aeruginosa*, SF- *Shigella flexneri*, SM- *Serratia marcescens*, ST- *Salmonella typhi*,
EF- *Enterococcus faecalis* and SA- *Staphylococus aureus*

Table 5 HPLC analysis of different *Aloe vera* accessions

Agro-climatic zones	Sample Name	RT (min.)	Area (Mau*s)	Yield (%)
Highland	Jammu	28.21	112.23	0.0826
	Himachal Pradesh (HP)	28.06	107.82	0.0793
Semi- Arid	Punjab	28.21	220.25	0.1621
	Haryana	28.91	129.11	0.095
Arid	Rajasthan	28.13	27.84	0.0205
	Gujarat	28.17	25.24	0.0186
Humid- Subtropical	Uttar Pradesh (UP)	28.13	154.15	0.1134
	Madhya Pradesh (MP)	28.17	80.35	0.0591
Tropical wet and dry	West Bengal (WB)	28.18	91.15	0.0671
	Andhra Pradesh (AP)	28.2	45.83	0.0337
Tropical wet	Goa	28.22	204.53	0.1505
	Kerala	28.25	99.88	0.0735
Standard	Aloe- emodin	28.1	135779	99

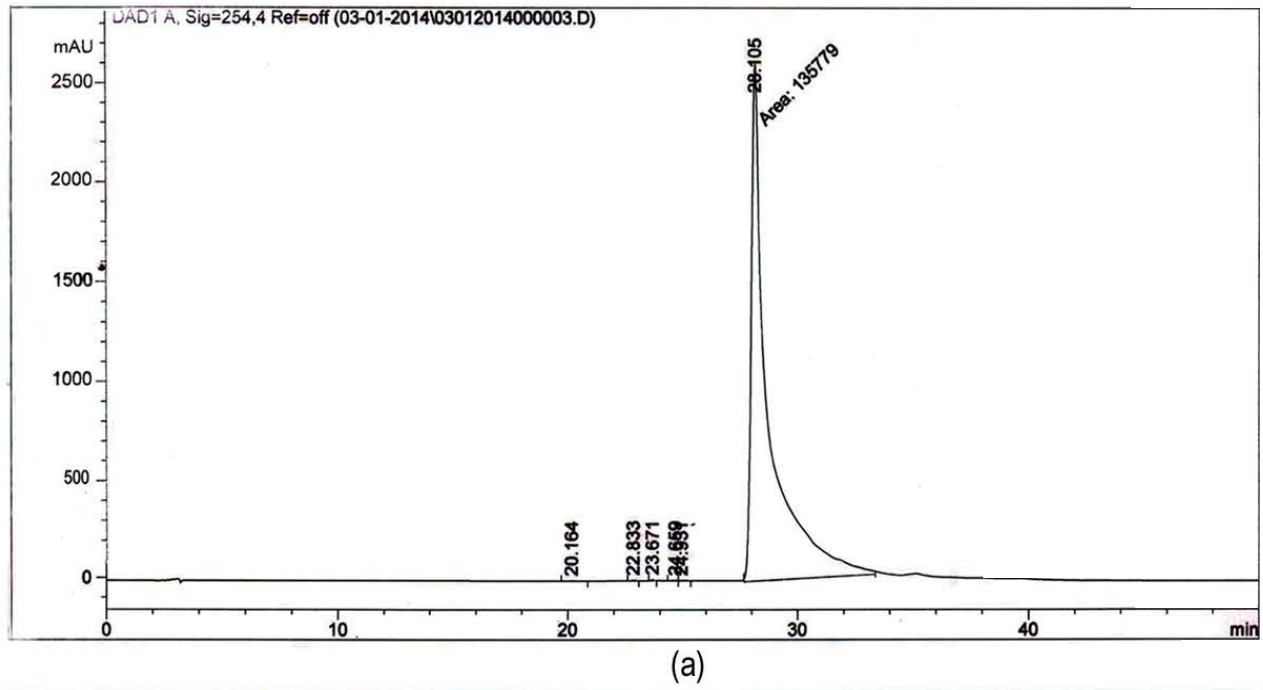

Figure 3a HPLC chromatogram of Standard

naturally occurring bioactive compounds anthraquinones also found in tropical and sub tropical areas throughout the world [47]. It supports the statement that subtropical environment conditions favor the anthraquinones production from the plants. It is known that genetic and environmental factors and their interactions affect the pharmaceutically important secondary metabolites in medicinal plants [48]. A variety of environmental factors; such as altitude, radiation, and soil nutrition; have been proven to significantly influence the secondary metabolite profile [49,50]. Furthermore, soil type, sun exposure, temperature and rain fall also have major effects on the phenol content of plants. The variations in the anthraquinone glycoside content could be affected due to the different altitude where the plant was grown [51].

Modern world is moving back towards herbal drugs. But standardization of herbal drugs with respect to concentration of its active principles is a must for their approval in modern medicine system. We have standardized an antimicrobial flavanol aloe- emodin from methanolic extracts of *Aloe vera*. Present study emphasizes that amount of aloe- emodin in extracts which may be related to the antimicrobial activity shown. Punjab, Haryana, UP and West Bengal showed significant correlation between percentage of presence of marker compound and antimicrobial activity. More marker antimicrobial compounds should be screened for formulation of an herbal drug with wider acceptability from *Aloe vera*. It will help in commercialization of plant in particular areas with desired pharmacological effects.

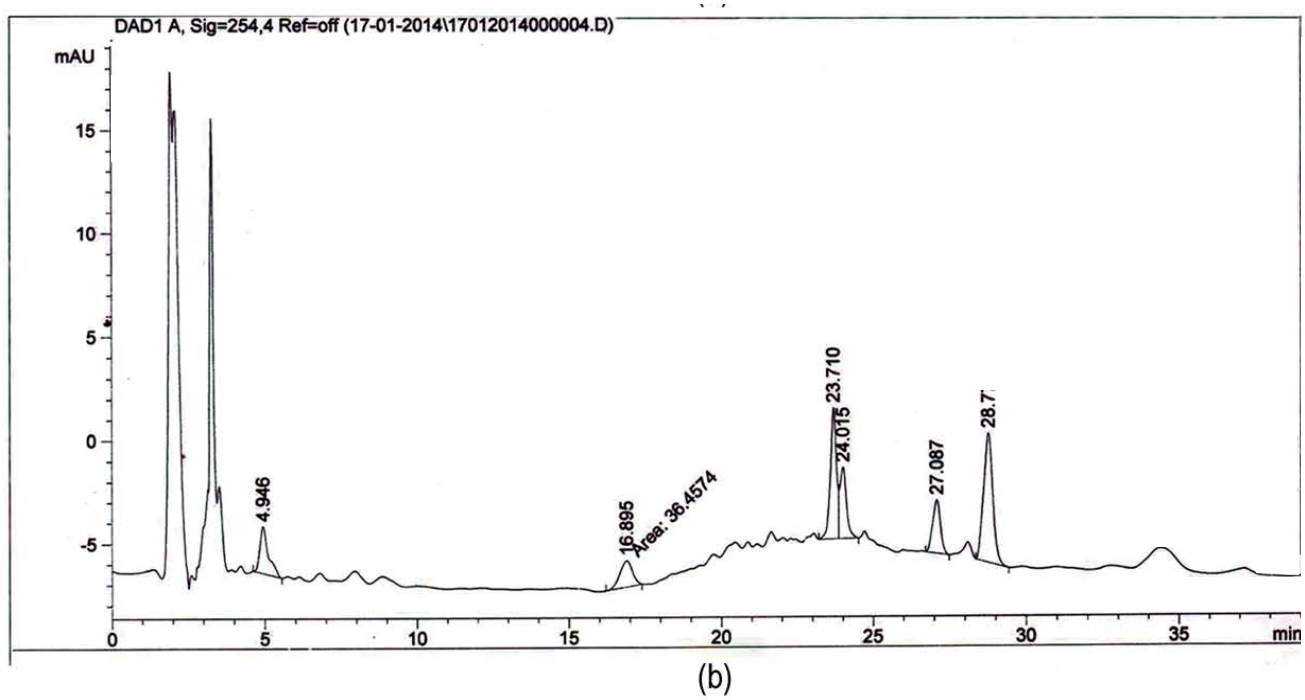

Figure 3b HPLC chromatogram of Gujarat accession

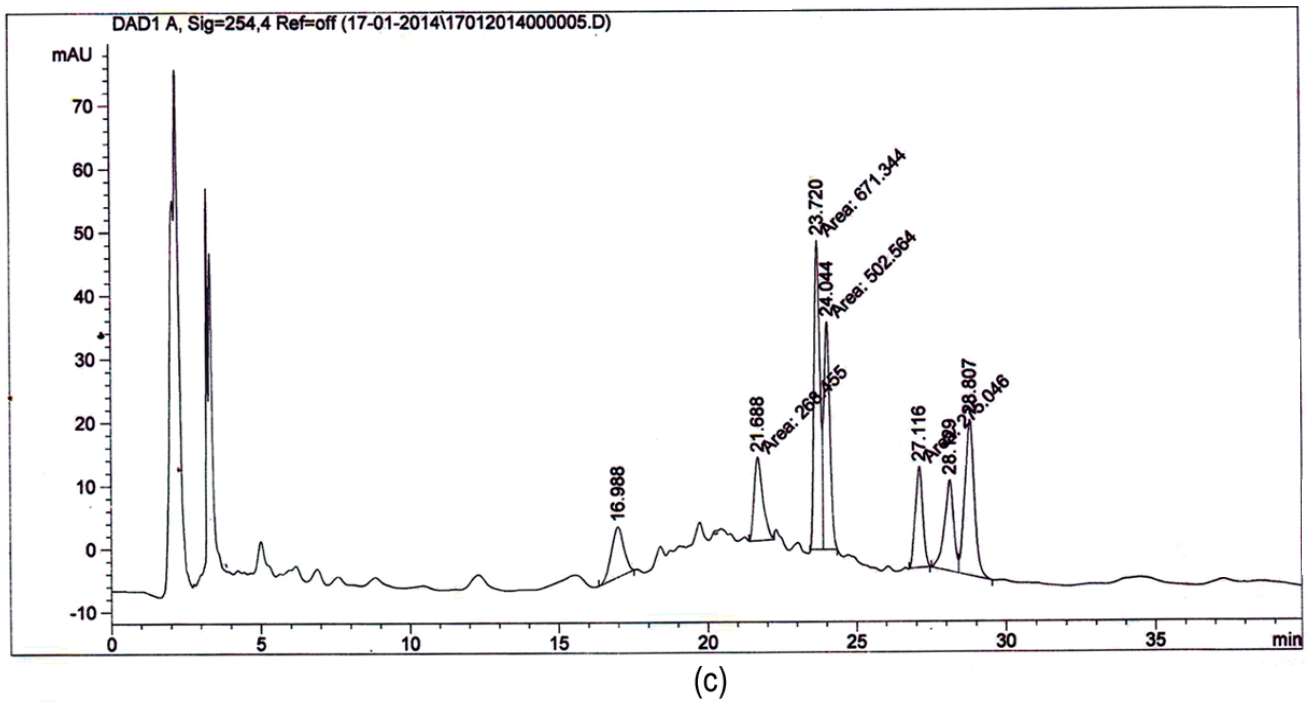

Figure 3c HPLC chromatogram of Uttar Pradesh accession

Conclusion

Aloe vera is a plant of great interest for the research purposes but most of works have been done on pharmaceutical level. Phytochemical composition of plants is influenced by various environmental and climatic factors. The present study has also showed the role of climatic conditions at different geographical locations on the presence of bioactive component aloe- emodin in crude extracts of *Aloe vera* due to which it may have good antibacterial activity. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

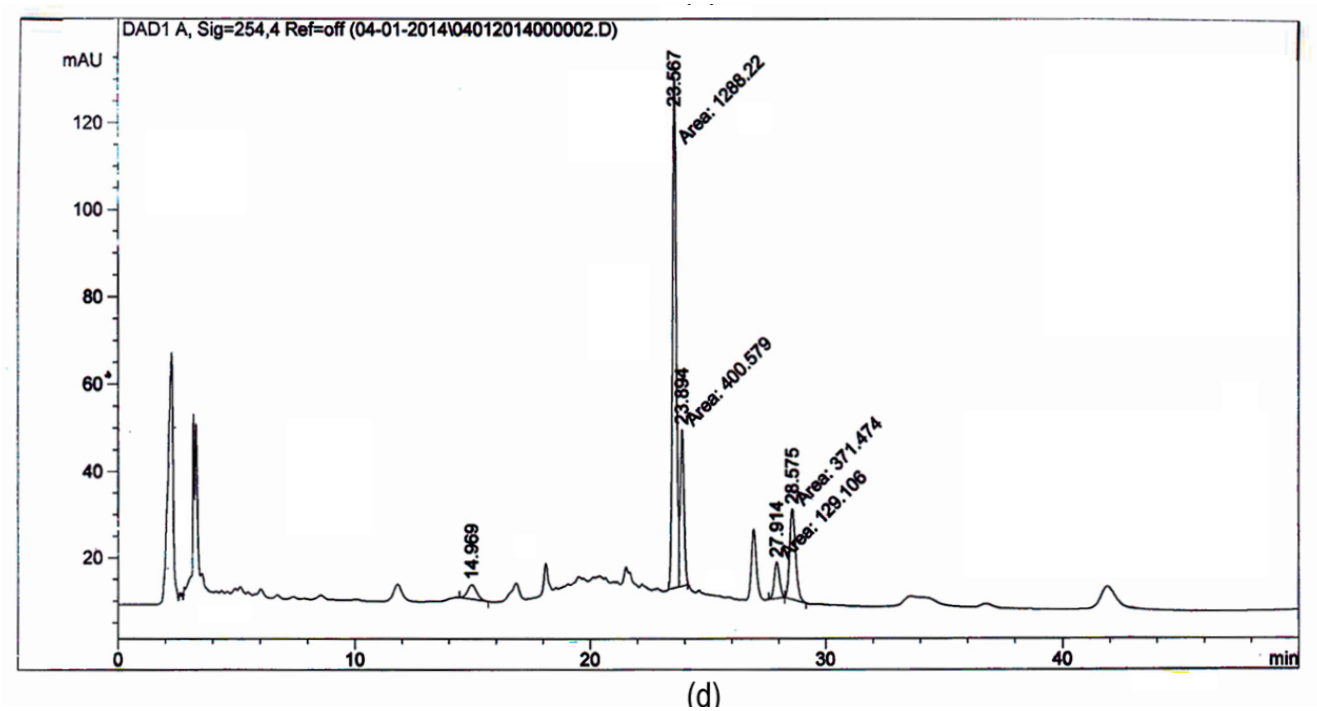

Figure 3d HPLC chromatogram of Uttar Pradesh accession

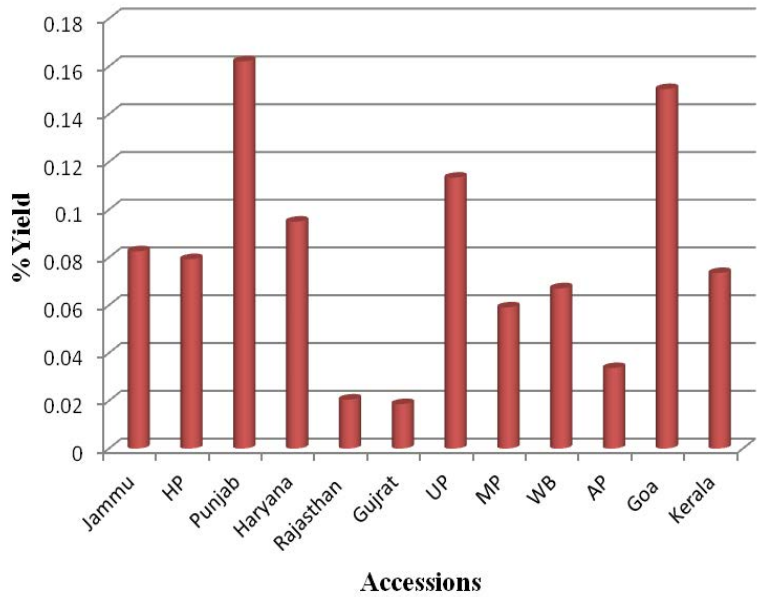

Figure 4 Showing the yield percentage of aloe-emodin from different accessions

Conflict of Interest

There is no conflict of interest between authors.

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