

Isolation and characterization of antimycobacterial compounds from the leaf of *Aloe vera* (L.) Burm. f.

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ABSTRACT

The aim of the present study was to evaluate the antimycobacterial activity of *Aloe vera* leaf, extracted in different solvents in increasing order of polarity and tested against *Mycobacterium smegmatis* (MTCC 994). Antimycobacterial activity was determined by agar well-diffusion method. Hot methanol extract showed the highest antimycobacterial activity (31 mm at 1000 mg/mL) and this extract was subjected to further purification. The purified fraction showed a zone of inhibition of 40 mm. The purity of the fraction was checked using HPLC and was subjected to NMR spectroscopy. Based on spectral data the active compound was identified as aloverose or acemannan.

INTRODUCTION

Mycobacterium tuberculosis, a facultative intracellular microbe belonging to the *M. tuberculosis* complex, is the most important cause of tuberculosis (TB), a chronic contagious disease caused by numerous species of mycobacterium in humans. Tuberculosis (TB) is the leading cause of deaths in adults and in 2002, the World Health Organization (WHO) estimated that between 2002 and 2020, approximately 1 billion people will be newly infected, over 150 million people will get sick, and 36 million will die of TB if control is not further strengthened. Moreover about one third of the world's population including 40 per cent from India was affected with TB (Gupta *et al.*, 2010). *M. tuberculosis* have developed resistance to the first line and second line drugs which has led to the emergence of Multi-Drug Resistant (MDR) and Extensively-Drug Resistant (XDR) strains all over the world. In view of this overwhelming

situation, there is an urgent need to search for alternative anti tuberculosis drugs, preferably those that can be readily and simply produced from natural plant species. Medicinal plants offer a great source to find original active drugs or new therapeutic agents (Chandran *et al.*, 2016) and hope to fulfill the needs for curing diseases plaguing human beings for many centuries. India is one of the few countries in the world which has unique wealth of medicinal plants and vast traditional knowledge of the use of herbal medicine for cure of various diseases. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts, the Vedas and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties (Hoareau and DaSilva, 1999). Only a very few plant species have been thoroughly investigated for their medicinal properties particularly against tuberculosis. *Aloe vera* is an important and traditional medicinal plant belonging to the family Liliaceae. It is widely used in herbal medicines and also in beauty and cosmetic products. This unattractive but highly beneficial plant has been used by different cultures since ancient times for its various benefits. The Egyptians referred to *A. vera* as the plant of immortality (Marshall 1990).

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Pharmacologically it is an immunity booster and detoxifies the system (Ro *et al.*, 2000). It is recommended in adjuvant therapy with antibiotics, Non steroidal Anti-Inflammatory Drugs (NSAIDs) and chemotherapy to eliminate drug induced gastritis and other adverse effects. It is also useful in various diseases such as type II diabetes, arthritis, eye disease, tumor, spleen enlargement, liver complaints, vomiting, bronchitis, asthma, jaundice and ulcers (Rajeswari *et al.*, 2012). However, from the above information it is well understood that although the extract of the plant is being used for asthma patients it has not been exploited against TB so far. Therefore, the present study was carried out to isolate and evaluate the antimycobacterial compounds from the extracts obtained from *Aloe vera*, using *Mycobacterium smegmatis* (MTCC 994) as reference sample, due to it being a fast grower, non-pathogenic and a simple model to work with. Moreover this species shares more than 2000 homologs with *M. tuberculosis* and shares the same unusual cell wall structure of *M. tuberculosis*.

MATERIALS AND METHODS

Chemicals and media

Chemicals for extraction, column chromatography (analytical grade) and methanol (HPLC grade) were purchased from Merck Limited, Mumbai, India. Silica gel (230–400 mesh) used for column chromatography and pre coated silica gel 60 GF₂₅₄ plates used for Thin Layer Chromatography (TLC) were purchased from Merck Limited, Germany. Microbiological media, Luria Bertani (LB) medium and Mueller-Hinton agar was procured from Hi-Media Laboratories Limited, Mumbai, India.

Bacterial strain

Mycobacterium smegmatis (MTCC 994) and was procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India and was used in the present study.

Plant material

Fresh and healthy *Aloe vera* leaves were procured from the local market, Thiruvananthapuram, Kerala, India. The *A. vera* leaves were washed, sliced and dried in a hot air oven at 50 °C for 72 h and powdered to 60 meshes in an apex grinder.

Preparation of extracts

The dried powder of *Aloe vera* leaves was subjected to hot and cold extraction using each of the solvents in the increasing order of polarity. About 100 g of dry *A. vera* leave powder was sequentially extracted using hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and water. After each solvent extraction step, the extracts were filtered and concentrated by using rotary evaporator (IKA, RV 10 digital, Germany). The percent extractive of hot and cold extracts of all the seven solvents was calculated using the formula.

$$\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Determination of antimycobacterial activity

In vitro antimycobacterial activity of seven extracts was tested against *M. smegmatis* by agar well-diffusion method (Mukherjee *et al.*, 1995). The extract which produced highest zone of inhibition were further purified using column chromatography and antimycobacterial activity was tested for 1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.8125 mg/mL concentrations. The overnight bacterial culture was resuspended in the saline to make the suspension 10⁵ CFU/mL and used for the assay. The plating was carried out by transferring bacterial suspension (10⁵ CFU/mL) to sterile petri plate and mixed with molten Mueller-Hinton agar medium and allowed to solidify. About 50 µl of the sample (5 mg/mL) was placed in the wells and allowed to diffuse for 2 h. Plates were incubated at 37°C for 48 h and the activity was determined by measuring the diameter of the inhibition zones. As the methanol extract showed highest antimycobacterial activity, it was selected for the isolation and purification of the bioactive compound.

Isolation of bioactive compound from methanol extract

Fractionation of the Methanol extract

Activated silica gel (60-120 mesh) was packed onto a glass column (450 × 40 mm) using n-hexane solvent. For the isolation of compound, 2.1 g of crude methanol extract was loaded on the top of silica gel. The column was eluted stepwise at a flow rate of 1 mL/minute and eluted successively with 200 mL of 100% hexane, 200 mL of linear gradient hexane: dichloromethane (v/v, 75:25 to 25:75), 200 mL of 100% dichloromethane, 200 mL of linear gradient dichloromethane: ethyl acetate (v/v, 95:5 to 5:95), 200 ml of 100% ethyl acetate and finally with 200 mL of 100% methanol. Two fractions (100 mL each) were collected from each combination.

TLC and HPLC

An aliquot of crystal compounds was loaded on the silica gel TLC plates (20 × 20 cm). The plates were developed using benzene: acetone (70: 30). The spots were located by exposing the plate to iodine fumes. The purified crystal compounds were tested for its purity using HPLC, using LC-10AT liquid chromatography (LC; Shimadzu, Singapore) equipped with a C-18 column (5 µm, 4.6 × 250 mm) and 100% methanol as a mobile phase with a flow rate of 1 mL/min. Ultraviolet (UV) detection was carried out with a diode array detector (Shimadzu).

Spectroscopic analyses of bioactive compound

The structure of the compound was determined using nuclear magnetic resonance (NMR) spectroscopy (Bruker DRX 500 NMR instrument, Bruker, Rheinstetten, Germany) equipped with a 2.5-mm microprobe. CDCl₃ was used as solvent to measure ¹H NMR experiment and the spectrum was recorded at 23°C. ¹H NMR spectra were recorded in CDCl₃ using tetramethylsilane (TMS) as internal standard at 500 MHz and the chemical shifts are given in parts per million and coupling constants in Hz. Chemical shifts are reported relative to the solvent peaks (CDCl₃: ¹H δ 7.24).

RESULTS

Sequential extraction of 100 g of *A. vera* leaves using hexane, chloroform, dichloromethane, ethyl acetate, acetone, ethanol and water yielded percentage extractives in the range of 0.27 to 4.40 %. The percentage extractive values of all the hot and cold extracts of different solvents are given in table 1. The highest percentage extractive of 4.40 % was obtained for aqueous cold extract and the lowest observed in cold acetone extract with 0.82 %. Among the seven extracts tested, *A. vera* leaf, the methanol extract showed the highest antibacterial activity and thus this extract was selected for further purification.

Table 1: Percentage extractives of *A. vera* in different solvents.

Extraction Process	Hexane	Chloroform	Dichloromethane	Ethyl acetate	Acetone	Methanol	Distilled water
Hot	1.33	1.6	0.41	0.32	0.82	1.9	3.28
Cold	0.79	0.27	0.53	0.61	1.28	1.01	4.40

The purification of methanol extract by silica gel (60 – 120 mesh) column chromatography yielded about twenty five fractions (Fr.1–Fr.25). Among these, fraction 5, 6 and 7 (Fr.5, Fr.6, Fr.7) showed positive activity in antimycobacterial assay, remaining fractions showed the lowest activity, respectively. Among the three positive bioactive fractions, fifth fraction showed highest activity (40 mm), hence selected for further purification using silica gel (230-400 mesh) column and

yielded 2 fractions (Fr_{m.1} and Fr_{m.2}), wherein 1st fraction (Fr_{m.1}) exhibited highest activity in antimycobacterial assay of about 25 mm zone of inhibition and other fractions did not show any inhibition. Extensively the first fraction (Fr_{m.1}) showed a single spot on TLC. The HPLC chromatogram of the fraction Fr_{m.1} is given in figure 1.

Table 2: Antimycobacterial activity of different *A. vera* extracts.

Solvent	Extract type	Concentration in mg/mL and zone of inhibition in mm (“-” No activity)							
		1000	500	250	125	62.5	31.25	15.625	7.8125
Hexane	Hot	-	-	-	-	-	-	-	-
	Cold	-	-	-	-	-	-	-	-
Chloroform	Hot	15	11	11	11	8	Nil	Nil	Nil
	Cold	-	-	-	-	-	-	-	-
Dichloromethane	Hot	-	-	-	-	-	-	-	-
	Cold	-	-	-	-	-	-	-	-
Ethyl acetate	Hot	-	-	-	-	-	-	-	-
	Cold	-	-	-	-	-	-	-	-
Acetone	Hot	-	-	-	-	-	-	-	-
	Cold	-	-	-	-	-	-	-	-
Methanol	Hot	31	23	19	19	18	17	17	13
	Cold	25	21	15	9	7	6	6	6
Water	Hot	-	-	-	-	-	-	-	-
	Cold	-	-	-	-	-	-	-	-

Pure compound was subjected to various spectral analyses. From the NMR data, the pure compound was identified as aloverose or acemannan. Aloverose is a partly acetylated polymannose which clearly showed a strong signal in δ 2.1 in ¹HNMR (Fig 2). The chemical structure of aloverose is given in figure 3.

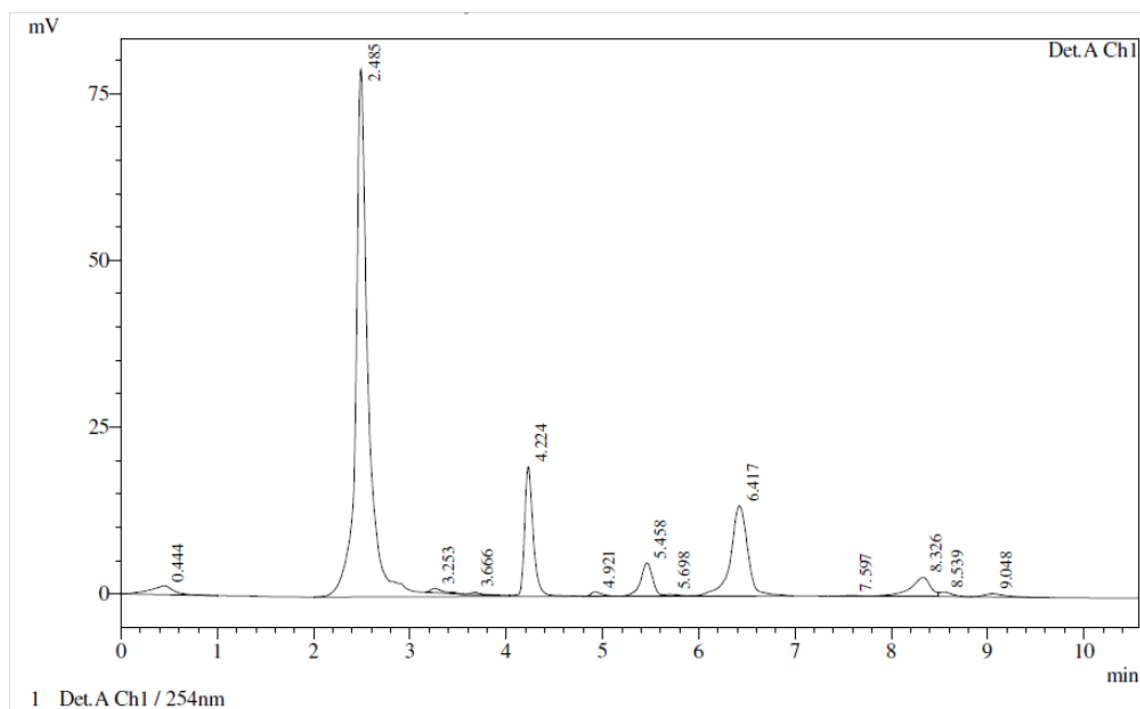


Fig. 1: HPLC chromatogram of the fraction Fr_{m.1}

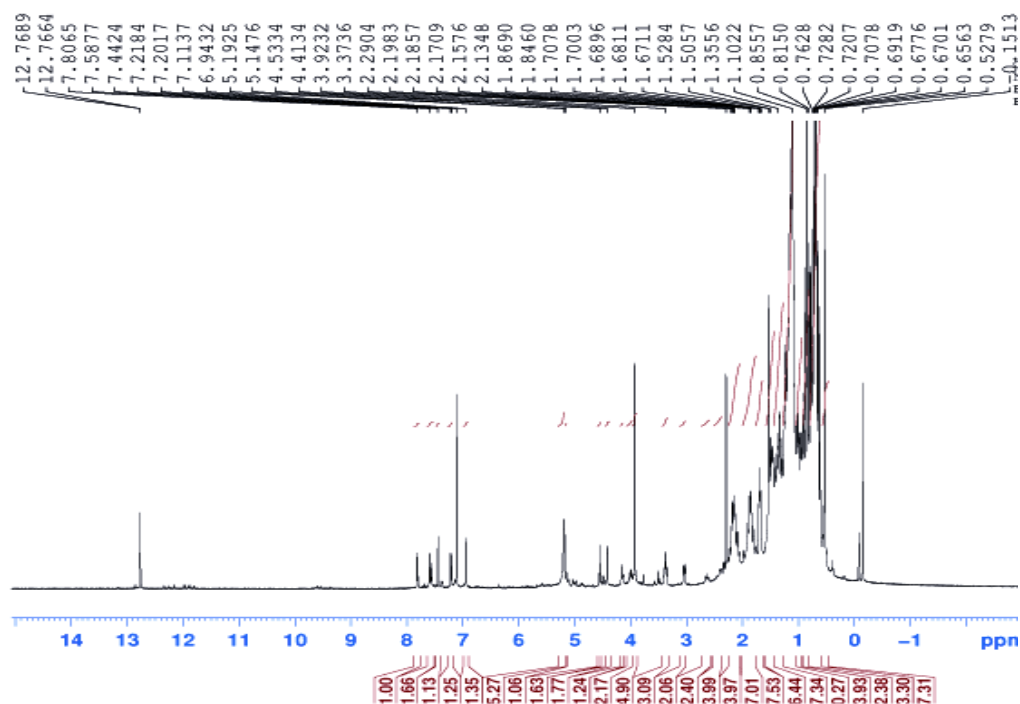


Fig. 2: ^1H NMR of aloverose.

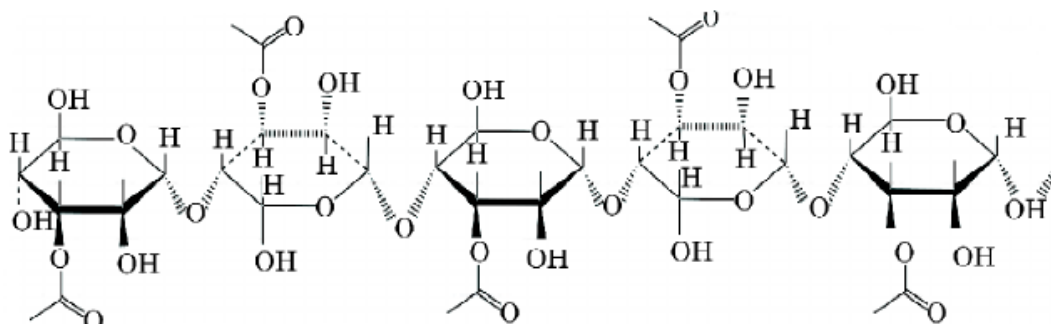


Fig. 3: Chemical structure of aloverose.

DISCUSSION

In the present study, the antimycobacterial activity of *A. vera* extract was evaluated and found that the hot methanol extract was very active against *M. smegmatis* with an inhibition zone of 31 mm at 1000 mg/mL. Mariita *et al.*, 2011 also reported similar observation that the aloe extract was highly active against three strains of *Mycobacterium* such as *M. fortuitum*, *M. smegmatis* and *M. kansasii* and a strong anti-mycobacterial activity against *M. tuberculosis* as well. Antimycobacterial activity of the *A. vera* juice against *M. smegmatis* were also reported by Alemdar and Agaoglu, 2009 and Thiruppathi *et al.*, 2010. Their findings are in line with the observations in the present study and the identification of active principle was not attempted by them.

Gupta *et al.*, 2010, reported that the aqueous extracts of *A. vera* was susceptible to MDR-TB strain, *Mycobacterium tuberculosis* H37Ra (MTCC 300). But in the present study the aqueous extract did not show any activity against *M. smegmatis*. *A. vera* is widely used in Ayurvedic, Homoeopathic and Allopathic systems of medicine and has been used to treat various skin conditions such as cuts, burns and eczema. In ayurvedic formulations, *A. vera* preparations are used as an appetite-stimulant, purgative, emmenagogue and antihelminthic, for treating cough, colds, piles, debility, dyspnoea, asthma and jaundice (Joseph and Raj, 2010). The plant leaves contains numerous vitamins, minerals, enzymes, amino acids, natural sugars and other bioactive compounds with emollient, purgative, antimicrobial, anti inflammatory, anti-oxidant, aphrodisiac, anti-

helmenthic, antifungal, antiseptic and cosmetic values for health care. *A. vera* has the potential to cure sunburns, burns and minor cuts, and even skin cancer. The external use in cosmetic primarily acts as skin healer and prevents injury of epithelial tissues, cures acne and gives a youthful glow to skin, also acts as extremely powerful laxative (Sahu *et al.*, 2013). The sap from *Aloe vera* is found to ease pain, reduces inflammation and helps in wound healing (Vogler and Enst, 1999).

The active components of aloe include anthraquinones, chromones, polysaccharides, and enzymes. The anthraquinones and chromones are responsible for the anti-cancer activity, anti-inflammatory, and evacuating (Choi *et al.*, 2001). The evidence also supports the use of *A. vera* for the healing of first to second degree burns (Maenthaisong *et al.*, 2007) and the wound healing property of *A. vera* gel has been attributed to Mannose-6-phosphate (Davis *et al.*, 1994).

Nejatzadeh-Barandozi 2013, reported that the methanol extract of *A. vera* gel extract contained phenolic acids/polyphenols, sterols, fatty acids, and indoles. Apart from these, various alkanes, pyrimidines, alkaloids, organic acids, aldehydes, dicarboxylic acids, ketones, and alcohols were also reported. More than 200 different constituents such as mucopolysaccharides, enzymes, sterols, prostaglandins, fatty acids, amino acids and a wide variety of vitamins and minerals were identified from *A. vera*. It contains several potentially active bioactive compounds including salicylates, magnesium lactate, acemannan, lupeol, campesterol, β -sitosterol, aloin A and anthraquinones (Ghosh and Playford, 2003).

The polysaccharides in *A. vera* gel consist mainly of linear chains of glucose and mannose molecules with considerably more mannose present than glucose. Alooverose (or Acemannan) is a β -(1,4)-linked galactomannan with acetylated mannose residues (Zhang and Tizard, 1996). Some of the pharmacological activities of acemannan include antiviral effects, wound healing acceleration, anti-cancer, and activation of macrophages and stimulation of T cells (Pugh *et al.*, 2001; Jia *et al.*, 2011; Lu *et al.*, 2003). However, in the present study, it was proved that the antimycobacterial activity against *M. smegmatis* was only because of the presence of alooverose in the plant extract.

CONCLUSION

The methanol extract of *A. vera* showed the highest antimycobacterial activity against *M. smegmatis*. The methanol extract was further purified, subjected to spectral analysis and after the spectral analysis; the active compound was identified as alooverose. The purified compound was also tested for antimycobacterial activity and this compound showed good antimycobacterial activity. It is concluded that alooverose found in *A. vera* leaf extract is a potent antimycobacterial agent which can inhibit the growth of *M. smegmatis*. This study is of high significance and giving an insight for the need of exploiting this

bioactive compound to test against *M. tuberculosis* (both MDR and XDR) strains.

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Conflict of Interests: There are no conflicts of interest.

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