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Evaluation of Bioactive Potential of an *Aloe vera* Sterol Extract

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We prepared a crude gel material from *Aloe vera* succulent leaf tissues. The ethanolic extract of lyophilized *A. vera* gel was used for the GC-MS analysis. Hexadecanoic acid (22.22%) was identified as major compound. Sitosterol and stigmasterol were found to be 2.89% and 2.1% in the extract. HPLC analysis was carried out to confirm the presence of stigmasterol. The concentration of sterol extract needed to scavenge DPPH free radical by 50% was calculated as 5.2 mg mL^{-1}. In the FRAP assay, the sterol extract showed significant hydroxyl radical scavenging in a dose-dependent manner (IC_{50} value 1.17 μg mL^{-1}). Concentration of the sample required to reduce lipid peroxidation was found to be 4.18 μg mL^{-1}, and the extract also possessed acetylcholinesterase activity (IC_{50} - 5.26 μg mL^{-1}). Catalase activity was 0.196 μM H_2O_2 decomposed min^{-1} μg^{-1} protein, whereas the peroxidase activity was 17.01 μM of pyragallol oxidized min^{-1} μg^{-1} protein. The extract recorded higher activity against growth of *S. greseus* and *C. albicans* in the experiments carried out to determine antibacterial and antifungal activity, respectively. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: Aloe vera; antimicrobial; antioxidant; sterol.

INTRODUCTION

Aloe vera has been used therapeutically since Roman times at least (Morton, 1961). The plant consists of solid components harboring over 75 biologically active compounds (Yim *et al*., 2011) which are known to have a broad range of pharmacological activities, including wound healing, anti-inflammatory, anti-arthritic, anti-oxidative, anti-diabetic, and anti-tumorigenic effects (Reynolds and Dweck, 1999; Boudreau and Beland, 2006). Additionally, *A. vera* plant is known to have antimicrobial properties (Reynolds and Dweck, 1999; Waihenya *et al*., 2002; Boudreau and Beland, 2006).

Although many pharmacologically important compounds have been identified and analyzed from *A. vera*, the literature is deficient in work on bioactive sterols from the plant. The sterols found in *A. vera* are cholesterol, sitosterol, campesterol, and lupeol. These sterols contain antiseptic and analgesic properties (Suga and Hirata, 1983). Recently, Misawa *et al*. (2012) observed amelioration of obesity associated metabolic disorders upon oral injection of *A. vera* sterols in diabetic rats.

Our laboratory is working on a long-term objective of over-expressing sterol desaturase gene for the recombinant production of stigmasterol in plant cells. As a preliminary study, we attempted to prepare a simple gel material from the succulent *A. vera* leaf tissues. We confirmed the presence of sterol in our ethanolic extract preparation by chromatography analysis and the various assays including antioxidant and antimicrobial were carried out. In this paper, we report our results on the bioactive potentials of a sterol extract from this pharmaceutically important plant.

MATERIALS AND METHODS

Plant material and preparation of gel. *A. vera* plants were obtained from Botanical garden, Tamil Nadu Agricultural University, Coimbatore, India and maintained in green house of Kongunadu Arts and Science College, Coimbatore, Tamil Nadu. The plant was identified from Botanical Survey of India, Coimbatore, India as *Aloe vera* (L.) Burm. f., and the certificate number was given as 'No. BSI/SRC/5/23/10-11/Tech.976'. Fresh leaves were collected and inner gel was collected by removing the outer skin and freeze dried. To prepare the extract, about 1 g of gel was dissolved in 10 mL of ethanol and incubated for 16 h at room temperature.

GC and HPLC analysis. The ethanolic extract of the sample was analyzed by GC-MS to identify the compounds. The crude ethanolic extract was further subjected to HPLC to determine the quantity of stigmasterol using commercial stigmasterol (Sigma-Aldrich, USA) as standard. The reversed phase HPLC system consisted of a pump equipped with a UV detector with C18 column. The mobile phase was acetonitrile (50): methanol (50). The injected volume was 20 μL. The flow rate was 1.5 mL min^{-1}. The spectrum was recorded at 230 nm.

Antioxidant assays. DPPH radical scavenging activity: The assay was performed according to the procedure of Blois (1958).

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FRAP assay: The ferric reducing ability of plasma (FRAP) assay was done by using the procedure of Benzie and Strain (1996). Ethanolic solutions of known Fe(II) concentration in the range of 50–500 M (FeSO4) were used to prepare calibration curve. The reducing power was expressed as equivalent concentration. This parameter was defined as the concentration of antioxidant having a ferric reducing ability equivalent to that of 1 mM FeSO4.

Hydroxyl radical scavenging activity: The assay was performed using the method as reported previously (Zhao *et al*., 2006).

Inhibition of lipid peroxidation: Lipid peroxidation assay was done as reported (Ruberto *et al*., 2000). However, we used 0.1 M of $FeSO_4$ instead of 0.07 M as described.

Anti-acetylcholine esterase assay: Anti-acetylcholine esterase activity was performed by using Ellman *et al*.'s method (1961) in a microtitre plate, using acetylcholine as substrate.

Antioxidant enzyme assays: Catalase and peroxidase enzyme assays were performed on *A. vera* sterol extract using the procedures of Rao *et al*. (1997) and Do *et al*. (2003), respectively.

Antimicrobial activity assays. The antibacterial activity of *A. vera* gel extract (AGE) was performed using the type cultures obtained from Kongunadu College Microbial Collection Centre (KCMC, Kongunadu Arts and Science College, Coimbatore, India) and Microbial Type Culture Collection Centre (MTCC, IMTECH, Chandigarh, India). The bacterial test organisms like *Bacillus cereus* MTCC 1272, *Salmonella typhi* MTCC 98, *E.coli* MTCC 443, *Staphylococcus aureus* MTCC 96, *Aeromonas hydrophila* MTCC 646, *Aeromonas salmonicida* KCMC 38, *Streptomyces tuiras* KCMC 60, *Streptomyces griseus* KCMC 61, and *Streptococcus* spp. KCMC 55 were used in the study. After the microbial lawn preparation, wells of 0.5 mm were prepared, and different concentrations of extract ranging from 10 to 100 μg was added. Ethanol was used as control. All plates were incubated at 27 °C for 24 h. The diameter of zone of inhibition of growth was measured in mm.

The fungal test organisms were obtained from KCMC (Kongunadu Arts and Science College, Coimbatore, India). The fungal strains like *Aspergillus flavus* KCMC 80, *Aspergillus terreus* KCMC 81, *Aspergillus niger* KCMC 82, *Penicillum* KCMC 83, and *Candida albicans* KCMC 84 were used in the study. Different concentrations of extract ranging from 10 to 100 μg was added in 0.5 mm wells in culture plate. The plates were incubated at 24 °C for 72 h. The diameter of zone of inhibition of growth was measured in mm.

Statistical analysis. The data obtained from antioxidant assay experiments were expressed as mean ($n = 5$), and the IC_{50} value was calculated using SPSS version 9.0. The data obtained from enzyme assays are expressed as mean ± standard deviation.

RESULTS

Extract preparation and chromatography analysis

Fresh *A. vera* leaves were collected, and the gel was taken out by removing the outer skin. After lyophilization, 131 g of gel yielded 2.44 g of lyophilized powder. The aqueous ethanolic extract was prepared due to the fact that phenolics are often extracted in higher amounts in one of the polar solvents like ethanol. GC-MS analysis was performed (Table 1) in which totally twelve compounds were identified. The well-known fatty acid hexadecanoic acid (22.22%) was identified as major compound followed by octadecenoic acid (16.2%), tricosane (5.59%) and 1-octadecanol (5.20%), sitosterol (2.8%), and stigmasterol (2.1%). To confirm the quantity of stigmasterol, HPLC analysis was done which showed positive result for the presence of stigmasterol. Stigmasterol was identified based on the retention frequency and average peak area when compared with standard stigmasterol (Sigma-Aldrich). The retention time of standard was found to be 3.25 and was compared with our extract which was found to be 2.09%, i.e. 2 g of stigmasterol is present in 100 g of sample.

Assay of antioxidant efficacy

The DPPH radicals scavenging activity of *A. vera* sterol extract showed concentration-dependent-free radical scavenging activity. The percentage reduction of the DPPH free radicals was observed in the range of 1.12 to 5.6%. The concentration needed to scavenge free radical by 50% was calculated as 5.2 mg mL^{-1}. A significant level of measured percentage of DPPH scavenging capacities noticed when increasing the concentration (mg mL^{-1}) of extracts at 5 min reaction. As expected, a higher percentage of DPPH scavenging is correlated to a higher antioxidant activity (Table 2).

FRAP value of the *A. vera* gel was determined. The level of scavenging activity of *A. vera* sterol extract was determined at 0 to 5th min. The reduction of a Fe^{+} ion in the assay, by the antioxidants in the samples, was monitored at 700 nm and the FRAP activities of

Table 1. GC-MS Analysis of ethanolic extract of *Aloe vera* gel

S. No.	RT	Name of the Compound	%
1.	4.68	Debocane, 4-methyl	2.37
2.	6.60	Trocosane	5.59
3.	13.\25	6-hydroxyhexane-3-1	2.61
4.	13.50	1-Dodecanol	3.05
5.	17.19	1-Octadecanol	5.20
6.	21.61	Hexadecanoic acid	22.22
7.	24.72	9-Octadecenoic acid	16.2
8.	25.16	Octadecanoic acid	4.99
9.	26.91	1-(Phenylthioxomethyl)piperidine	3.09
10.	29.56	Docosane	3.27
11.	38.78	Sitosterol	2.89
12.	36.10	Stigmasterol	2.1

Table 2. Inhibition of DPPH radical by *Aloe vera* sterol extract

Concentration (mg mL^{-1})	DPPH Scavenged (%)	IC_{50} mg mL^{-1}
1.12	13.59 ± 0.35	5.2
1.4	18.47 ± 0.64	
2.8	29.82 ± 0.48	
4.2	40.36 ± 1.09	
5.6	56.81 ± 1.53	

the samples expressed which was found to be 403 μM mg^{-1}. A significant hydroxyl radical scavenging activity was observed in a dose-dependent manner. The inhibition percentage ranged between 67.9 and 93.01, and the IC_{50} value was calculated as 1.17 μg mL^{-1} (Table 3).

The sterol extract inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenate was dose dependent. The inhibitory percentage was monitored at 532 nm and recorded in the range of 73.7 to 97.1%. The concentration of the sample required to reduce the lipid peroxidation was calculated as 4.18 μg mL^{-1}. Anti acetylcholine esterase activity of the extract was performed at various concentrations and the percentage of inhibition was found between 67.8 and 92.6%, and the IC_{50} value was calculated as 5.26 μg mL^{-1}. Catalase activity exhibited by the extract was 0.19 ± 0.09 μM H_2O_2 decomposed min^{-1} μg^{-1} protein. The activity of peroxidase in the extract was monitored at 430 nm for 3 min and recorded 17.0 ± 0.74 μM of pyragallol oxidized min^{-1} μg^{-1} protein.

Antimicrobial activity

The inhibitory activity of sterol extract was performed against nine bacterial cultures, and the zone of inhibition was observed and the diameter of minimum zone of inhibition was measured. Significant level of susceptibility was recorded in all the concentrations tested. Higher inhibitory activity in lower concentration (10 μg mL^{-1}) of extract was recorded with *S. typhi* and *S. griseus*. Less significant inhibitory activity was recorded against *E. coli* and *B. cereus* (Table 4). It was also found that the sterol extract is able to inhibit growth of *C. albicans* and *A. terreus* (Table 5).

DISCUSSION

Aloe gel yield obtained in the study was approximately 18 mg of leaf tissue suggesting the efficiency of this simple method for obtaining the bioactive gel material. Aloe gel has been reported as colorless gel enclosed in innermost of the fresh leaves (Reynolds and Dweck, 1999). The gel is reported to consist primarily of water (>98%) and polysaccharides, i.e. pectins, cellulose, hemicellulose glucomannan, acemannan, and mannose derivatives), sterols, aminoacids, vitamin C, and B complex (Bozzi *et al*., 2007). Saccu *et al*. (2001) reported the major active constituents of aloe latex as hydroxyanthracene derivatives (15 to 40%) such as the anthraquinone glycosides aloin A and B. Many of the medicinal effects of aloe leaf extracts have been attributed to the polysaccharides found in inner leaf parenchymatous tissue (Ni *et al*., 2004), but it is believed that these biological activities should be assigned to a synergistic action of the compounds contained therein rather than a single chemical substance (Dagne *et al*., 2000). Misawa *et al*. (2012) suggested that aloe sterols could be beneficial in preventing and improving metabolic disorders with obesity and diabetes in rats.

In their GC-MS analysis, 26 bioactive phytochemical compounds were identified in the ethanolic extract of *A. vera*. However, in our study totally, 12 compounds were identified including hexadecanoic acid, octadecanoic acid, tricosane, 1-octadecanol, and trace amounts of sterols. Our HPLC results with standard stigmasterol confirmed the presence of pharmacologically important stigmasterol in our ethanolic extract. Sterols are one among the important agents having wealthy anti-inflammatory effects. These sterols contain antiseptic and analgesic properties. They also have

Table 3. Hydroxyl radical scavenging activity of *Aloe vera* gel

Concentration (μg μL^{-1})	% of inhibition	IC_{50} μg mL^{-1}
10	67.9 ± 0.73	1.17
20	70.4 ± 0.81	
30	80.34 ± 1.25	
40	92.56 ± 0.95	
50	93.01 ± 0.50	

Table 4. Antibacterial activity of *Aloe vera* sterol extract

	Diameter of zone of growth inhibition (mm) in various concentration of *Aloe* gel (μg mL^{-1})				
Bacteria	10	20	30	50	100
S. typhi	10.0	12.0	13.0	17	17.6
E. coli	4.0	8.0	11.0	12.0	14.0
A. hydrophilla	8.0	18.0	21.0	24.0	28.6
A. salmonicida	8.0	12.0	16.0	10.0	14.0
Streptococcus spp.	8.5	20.0	18.7	18.8	24.0
S. aureus	8.6	10.3	10.6	12.4	15.0
B. cereus	4.8	8.8	10.0	10.6	14.0
S. tuiras	8.0	10.0	10.0	24.0	28.0
S. griseus	10.0	18.0	10.6	28.6	24.0

Table 5. Antifungal activity of *Aloe vera* sterol extract

	Diameter of zone of growth inhibition (mm) in various concentration of *Aloe vera* sterol extract (μg mL^{-1})				
Fungi	10	20	30	50	100
A. terreus	4.0	6.0	11.0	13.0	15.0
Penicillium sp	10.0	8.0	11.0	14.0	18.0
C. albicans	11.0	8.0	4.0	15.0	25.0
A. niger	8.0	10.0	18.0	13.0	15.0
A. flavus	5.0	4.0	14.0	15.8	16.0

pain-killing properties similar to aspirin (Suga and Hirata, 1983).

Plants produce antioxidants as secondary metabolites. They are mainly phenolic which serves in plant defence mechanisms to counteract reactive oxygen species in order to avoid oxidative damage (Velioglu *et al*., 1998). Our DPPH radical scavenging activity assay showed the activity in concentration dependent manner. When increasing the concentration of the extracts, a significant level of DPPH scavenging activity was observed. Hydroxyl scavenging activity assay also yielded similar results indicating the high antioxidant potentials of our extract preparation. Sultana *et al*. (2009) investigated the antioxidant activity of *A. barbadensis* leaves from the four extracting solvents [absolute ethanol, absolute methanol, aqueous ethanol (ethanol:water 80:20 v\v) and aqueous methanol (methanol:water,80:20 v\v)] and by two extraction techniques (shaking and reflux) and found that, it has 37.2 to 86.6% DPPH scavenging activity.

The effect of oral feeding of 2% AGE for 30 days on azoxymetahne (AOM)-induced oxidative stress in rats has been reported (Anilkumar *et al*., 2010). It was observed that there was significant raise in hepatic catalase, superoxide dismutase, and glucose-6phosphate dehydrogenase activities as a result of feeding of the extract. Hence, they suggested that AGE possesses the ability to reduce AOM-induced oxidative stress and toxicity in liver. Acetylcholinesterase, an important component of cholinergic synapses, colocalizes with amyloid-β peptide deposits of Alzheimer's brain. Hence, the anti acetylcholineesterase activity observed in the present study has a greater potential of further exploitation in the pharmacological studies.

A basic peroxidase has been identified in commercial gel of *A. barbadensis. In vivo*, the activity is localized in the vascular system of inner aqueous leaf parenchyma (Esteban *et al*., 2000). Our observations in *A. vera* are agreeable with the previous reports of peroxidase activity in *A. barbadensis*, and, in addition, the sterol extract possessed significant catalase activity.

About nine of the human pathogenic bacteria were used in the antibacterial screening. Although the extract inhibited the growth of all the test bacteria, greater effect was observed against *S. greseus*. Lawrence *et al*. (2009) found that ethanol and methanol extracts showed higher activity, while acetone extract showed least or no activity against most of the tested pathogens. Since our extract is also made using ethanol as solvent, it is evident that the compounds identified in our study might have had antibacterial properties.

Dried latex (*Aloe* drug) has been reported to show higher activity against *Colletotrichum* species than *Fusarium solani*. Two constituents, namely aloin and aloe-emodin, were identified as active principles by their activity against *C. gloeosporides* and *Cladosporium cucumerinum* (Sebastian *et al*., 2011). All these previous studies were done to test antifungal activity against plant pathogenic fungi. Our study represents the first report, to our knowledge, on the antifungal activity of *A. vera* sterol extract against human pathogenic fungi. We believe that our study on sterol extract of *A. vera* contributes to our existing knowledge on therapeutic properties of this medicinal plant.

Conflict of Interest

The authors have declared that there is no conflict of interest

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