INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF *ALOE VERA* L

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Article Received on: 17/08/12 Revised on: 01/09/12 Approved for publication: 13/10/12

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ABSTRACT

The present study was made to attempt the antimicrobial and phytochemical analysis of *Aloe vera* L (babosa). The antimicrobial activity method was using Muller and Hinton agar Dimethyl sulfoxide (DMSO) was used. The Overnight incubated bacterial culture, *Staphylococcus aureus, Bacillus subtillis, Proteus vulgaris, Pseudomonas aeroginosa, Enterobacter aerogenes, Klebshiella sp, Salmonella sp, Shigella sonie, S. spidermiods*. In phytochemical studies, the leaf extract were analysed for the flavonoids, pholabatannis, glycosides, phenols, catachol, resins, saponins, lipids and fats, tannis, acidic compounds, terpenoids, reducing sugars, anthraquinone, carbohydrates, steroids, and sterols etc. In analysis of Tannin compounds brownish green colour developed to indicate the presence of Tannin. In this screening process Tannin, Saponin, Flavonoids and Terpenoids compounds revealed positive results

Antibacterial activity of *A.vera* was analysed against *E.coli, Enterobacter aerogens, Staphylococcus sp, Proteus mirabilus, Pseudomonas sp., Shigella sonie, Salmonella sp, S. spidermiods, Klebshiella sp*. Among the three bacterial organisms maximum growth suppression was observed in *Staphylococcus sp, Enterobacter aerogens* and *Klebsiella sp*. Anti bacterial activity of *A.vera* was analysed against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa and E. coli. A.vera leaf gel can inhibit the growth of two gram positive bacteria Shigella flexneri* and *Streptococcus pyogenes*. Specific plant compounds such as anthroquinones and dihydroxy anthroquinones as well as Saponins have been proposed to have direct antimicrobial activity.

The antioxidant activity of *A.vera* leaf and gel extract by using DPPH free radical scavenging assay method. The antioxyactivity of *A.vera* leaf and gel aqueous extract was determined at the concentration (100, 200, 300, 400 and 500 µg/ml) and IC 50 was calculated. In, DNA protective activity of *A.vera* on blood DNA against free radicals generated by H_2O_2. The results indicator that plant leaf extract had maximum DNA protective activity than gel extract against free radicals. The present study *A.vera* gel and leaf extract was analysed by HPLC chromatogram and should the presence of glucomannose, galactoglucoaralimannone and gluconic acid, vitamin C and also anthraquinone, phenols, and chromones.

Keywords: Antimicrobial, Phytochemical, *Aloe vera* L, HPLC

INTRODUCTION

Aloe vera (*Aloe barbadensis* Miller) is part of the liliaceae family and is a perennial succulent cactus plant, which grows in hot and dry climates [5]. It has been used in the traditional medicinal practices and in the treatment of a variety of disorders including wounds and burns[9] It is often referred to as a healing plant and is the sources of two products. First one is bitter yellow juice from cut leaf base, which contains a high concentration of antraquinone compounds and when dried is used as a potent cathartic[12] and lacquer to inhibit nail biting. The second is mucilaginous juice from the leaf parenchyma, which has been used as a remedy for a variety of pathological states, such as arthritis, gout, acne, dermatitis, burns and peptic ulcers induced by epithelial alterations[4,19] It was reported to have anti-inflammatory and analgesic activities by inhibiting pain-producing substances such as bradykinin or thromboxane and cyclooxgenease[3,20] antibacterial and antifungal properties[2,18]. Polysaccharides are another group of juice constituents to which activity has been ascribed, particularly in immunodulatory reactions[1]. It is also claimed to have hepatoprotective, antiproliferative, anti-carcinogenic, antiaging, and leaxative effects [21]. These effects are thought to be the result of radical scavenging and immuno-modualtory mechanisms. *Aloe vera* is thus incorporated into many products and is used for various medicinal, cosmetic and nutraceutical purposes with topical applications features prominently. The aim of the present study was undertaken the antimicrobial and phytochemical analysis of *Aloe vera* L

MATERIALS AND METHODS

Plant Materials

Aloe vera L (babosa) leaves were collected and processed from a single garden plant, to obtain a fresh extract for each experiment during this work .The leave samples were collected from the Bala Nursery Garden, Muthanompatti, Allampatti, Virudhunagar, Virudhunagar district. Tamilnadu, India. The selected plant leaves were subjected to extraction method. The extraction method was done by the suitable procedure [6].

Bacterial Strains

The Overnight incubated bacterial culture, *Staphylococcus aureus, Bacillus subtillis, Proteus vulgaris, Pseudomonas aeroginosa, Enterobacter aerogenes, Klebshiella sp, Salmonella sp, Shigella sonie, S. spidermiods*.

Preparation of crude extracts

Aloe barbadensis miller latex was cut into small pieces and homogenized. The homogenized plant material was extracted with ethanol (95%). The extract (ethanol) was evaporated at 45°C temperature under reduced pressure to a syrup like residue. The solvent was completely removed and dried ethanol was re-dissolved in different concentrations in their respective solvents of ethanol. The present yield of each extract was calculated [10].

Disc Diffusion Assay for microbial sensitivity testing

Agar disc diffusion assay for screening the antibacterial potential of ethanol extract The disc diffusion assay methods[14,18]. The total activity (µg/ml) was calculated [6].

HPLC

HPLC was performed using a TSP system (Thermo Quest, Tokyo, Japan) equipped with an AS 4000 autosampler (injector pushloop with a flush volume of 400µL), two P4000 gradient pumps, a UV 6000 photodiode array detector (200-500 nm range: 5nm bandhwidth) and an LCQ ESI/ MS detector controlled by chromoquest software(version 2.51; 1998). Analyse were performed using a Hypersil Hipurity (Thermo Hypersil, Kleinostheim, Germany) C_{18} coloumn (150X4.6mm). The mobile phase comprised acetonitile (solvent A) and water (solvent B) with both solvents containing 0.02% trifluoroacetic acid. The gradient

programme commenced at 95:5 (V/V) of A:B and for 25min to 60:40 (A:B). The total running time was 5min with a flow rate of 1.2 mL/min. Samples were prepared by dissolving 5mg of the lyophilized Aloe extract in 1mL 30% (v/v) aqueous ethanol. The sample size for analysis was 20µL.

Preparation of Hydrogen per oxide assay

Hydrogen per oxide occurs naturally at low concentration in the air, water, human body, plants, microorganism, food and beverages. Human beings are continuously exposed to H_2O_2 indirectly via the environment which is estimated as 0.28mg/hg/day. Hydrogen per oxide enters the human body through inhalation of vapour or mist and through eye or skin contact. In body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH) that can initiate lipid peroxidation and cause DNA damage. [7]

Isolation of Blood DNA

200µl of the given blood sample was taken. Double the volume of SSC buffer was added and centrifuged at 5000rpm for 5mins. Supernatant was removed, which contains plasma blood proteins and other components. Double the volume of 0.2M sodium acetate was added to the pellet. 50µl of 10% SDS was added and 1ml of phenol: chloroform : isoamylalcohol was also added and mixed gently. Clear brown solution was appeared. Then, it was centrifuged at 10,000 rpm for 10minutes. The aqueous phase was transferred to a fresh tube and 500µl of chloroform: iso amyl alcohol was added and centrifuged at 10,000 for 10minutes. The aqueous phase was transferred to a fresh tube and 500µl of 100% ethanol was added and centrifuged at 10,000 rpm for 10minutes. The supernatant was discarded the pellet was washed with the 75% ethanol at 10,000 rpm for 10minutes. The supernatant was discarded and the DNA pellet was dried and resuspended in TE buffer. The genomic DNA was separated in 1% agarose gel electrophoresis.

Agarose Gel Electrophoresis

Separation of isolated genomic DNA by Agarose Gel Electrophoresis

- ✓ 15µl of DNA was loaded in 0.8% Agarose gel.
- ✓ The DNA was separated and visualized with the UV illuminator.

Radical scavenging activity – DPPH assay [16].

Six different ethanol dilutions of *A.vera* plant leaves extracts (10, 20, 30, 40, 50, 100µg/ml) were mixed with 1ml of 0.2mM ethanolic solutions of DPPH. Ethanol (1ml) plus plant extract solution was used as blank. The absorbance was measured at 518nm after 1hour of reaction at 37˚C. DPPH was prepared daily and protected from light. Scavenging capacity in percent (IC %) was calculated using the equation:

IC% = [(A blank- A sample)]/ A blank X 100

Where A sample is the absorbance obtained in the presence of different extract concentrations and A blank is that obtained in the absence of extracts. Tests were carried out in petriplate.

Qualitative and phyto-chemical analysis of plant extracts

The leaf extract were analysed for the flavonoids, pholabatannis, glycosides, phenols, catachol, resins, saponins, lipids and fats, tannis, acidic compounds, terpenoids, reducing sugars, anthraquinone, carbohydrates, steroids, and sterols etc., as follows Alkaloids, carbohydrates, tannins and phenols flavonoids, gums and mucilages, phytosterol, proteins and amino acids, fixed oils and fats, volatile oil and saponins were qualitatively analysed. Qualitative phytochemical analysis were done by using the procedures [15, 22] In quantitative analysis, Alkaloids, Tannins and Phenols, Free amino acids Flavonoids were determined. Determination of Amino acids by Chromatograpic method using a method[13]. The biochemical parameters like Total phenols, Total alkaloids, β-sitosterol and flavanoids [15] were quantitatively estimated.

Table 1: Phytochemical analysis of *Aloe vera* samples

Phytochemical analysis	Leaf	Gel
Flavonoids	+	-
Saponin	+	-
Phlobatannis	-	-
Resins	+	-
Sterols	-	+
Steriods	-	-
Tannins	+	-
Glycosides	+	-
Acidic compounds	+	-
Reducing sugars	-	+
Carbohydrate	-	+
Anthraquinone	+	-
Terpenoids	+	-

+positive - negative

Table 2: Antimicrobial activity of *A.vera* leaf and gel

Organism	Leaf(mm)*	Gel(mm)*
E.coli,	5.0 ± 0.012	10 ± 0.54
Enterobacter aerogens	13.5± 0.054	18± 0.45
Klebshiella sp	13.2± 0.067	17 ± 0.56
Proteus mirabilus	5.2± 0.034	`9 ± 0.67
Pseudomonas sp.,	4.56 ± 0.056	9 ± 0.78
S. spidermiods	4.54 ± 0.067	7 ± 0.78
Salmonella sp	12.3 ± 0.23	15 ± 0.34
Sigella sonie	4.2 ± 0.012	6 ± 0.23
Staphylococcus sp	12.4 ± 0.098	19 ± 0.98

* All the values are the averages of five observations (Mean ± SE) mm-millimeter

Table 3: Antioxidant activity of *Aloe vera* plant extract DPPH assay method

Concentration (µg/ml)	% of Inhibition*
100	83±0.054
200	76±0.067
300	65±0.089
400	50±0.056
500	44±0.067

* All the values are the averages of five observations (Mean ± SE)

Table 4: Antioxidant activity of *Aloe vera* plant gel extract DPPH assay method

Concentration µg/ml	% of Inhibition*
100	81± 0.078
200	70±0.056
300	62±0.078
400	42±0.034
500	31±0.078

* All the values are the averages of five observations (Mean ± SE)

Table 5: DNA protective activity of *Aloe vera* plant leaf extract (Ethanol extract)

Lane	1	2	3	4	5
Sample	DNA	DNA + H_2O_2	DNA + H_2O_2 + Leaf extract	DNA + H_2O_2	DNA + H_2O_2 + Gel extract

Table 6: Peak value of *Aloe vera* leaf through HPLC

Peak value	Name of the compound
38.73	Anthraquinone
33.12	Semi anthraquinone like derivatives
30.09	Vitamins B complex
24.46	Unknown components (may be enzymes or organic acid)
22.76	Phenol-chromones
18.01	Polysaccharides
17.41	Lignin
2.50	Chromones

Table 7: Peak value of *Aloe vera* gel through HPLC

Peak value	Name of the compound
41.6	Glucomannone
38.71	Acetylated mannan
33.1	Pentose derivatives
27.63	Monosaccharides (alverose)
17.33	Acetylated derivatives
14.79	Galactoglucoarabinomannan
3.16	Glucuronic acid

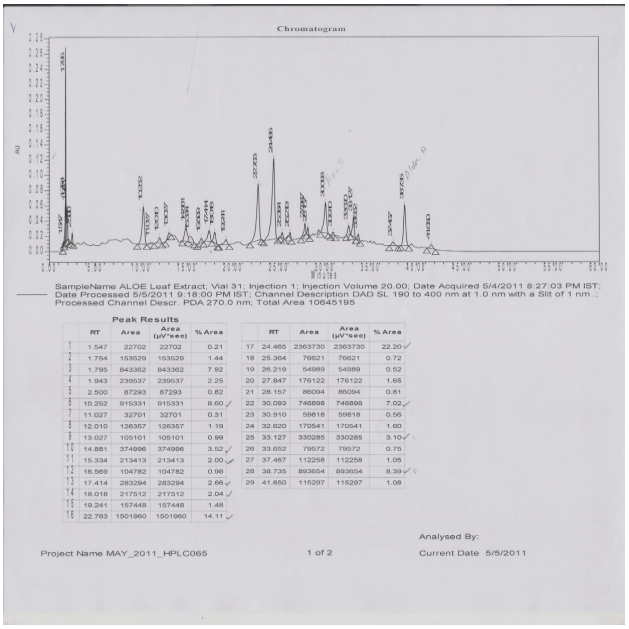

Figure 1: Peak value for leaf through HPLC

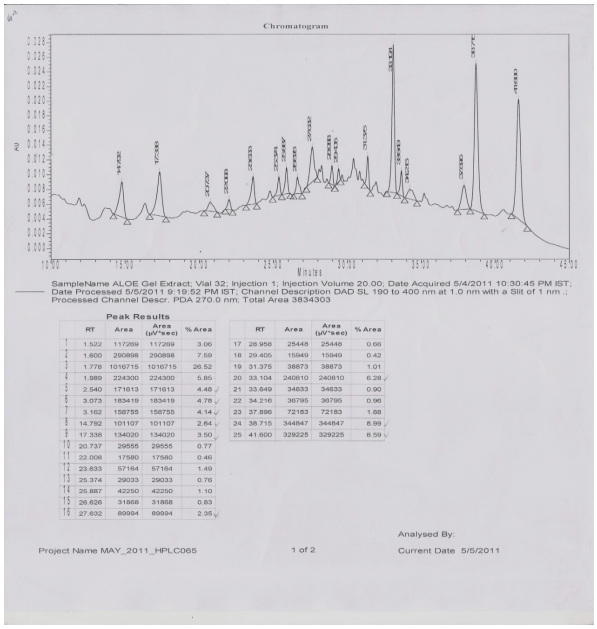

Figure 2: Peak value for gel through HPLC

RESULT AND DISCUSSION

The present study carried out on the *A.vera* revealed the presence of medicinal active constituents. The phytochemical active components of *A.vera* were qualitatively analysed and the results are presented in Table-1. In analysis of Tannin compounds brownish green colour developed to indicate the presence of Tannin. Similarly based on the presence or absence of colour change indicates positive and negative results. In this screening process Tannin, Saponin, Flavonoids and Terpenoids revealed positive results in the plant extracts.

Antibacterial activity of *A.vera* was analysed against *E.coli, Enterobacter aerogens, Staphylococcus sp, Proteus mirabilus, Pseudomonas sp., Sigella sonie, Salmonella sp, S. spidermiods, Klebshiella sp*. Among the three bacterial organisms maximum growth suppression was observed in *Staphylococcus sp, Enterobacter aerogens* and *Klebsiella sp* results are presented in Table-2.

Anthraquinones may act as antioxidants and radical scavengers. Reactive oxygen species and free radical mediated reactions are involved in inflammatory responses and can contribute liver necrosis [8].

The models of scavenging the stable DPPH radical are widely used to evaluate the free radical scavenging ability to various samples. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517nm induced by antioxidants. The extracts were able to reduce the stable radical DPPH to the yellow-colored diphenyl picryl hydrazine. The scavenging effects of *A.vera* gel methanol extract showed better radical scavenging activities [16].

A present study was carried out to test the antioxidant activity of *A.vera* leaf and gel extract by using DPPH free radical scavenging assay method. The antioxyactivity of *A.vera* leaf and gel aqueous extract was determined at the concentration (100,200,300,400,500 µg/ml) and IC 50 was calculated (Table-3 , 4).

Carcinogenesis induced by DNA adduct formation was shown to be inhibited by a polysaccharide rich aloe gel fraction in an *in vitro* rat hepatocyte model chemo preventive effect of aloe polysaccharide isolated from *A.vera* nothing that oxidative DNA damage assessed by 8 – hydroxyguanosine was significantly reduced by the polysaccharide, which also inhibited benzo[a]pyrene-DNA adduct formation by interfering with benzo[a]pyrene-DNA absorption *in vivo*. This may be due to the inhibition of carcinogen activation systems or to the induction of detoxifying enzymes. A present study was carried out to test the DNA protective activity of *A.vera* on blood DNA against free radicals generated by H_2O_2. The results indicator that plant leaf extract had maximum DNA protective activity than gel extract against free radicals as shown in Table-5. Similar results was reported [23].

The present study *A.vera* gel and leaf extract was analysed by HPLC chromatogram (Figure 1 and 2) and should the presence of glucomannose, galactoglucoaralimannone and gluconic acid, vitamin C and also anthraquinone, phenols, and chromones. A study by . Grindly and Reynolds, (1986) [9] reported on a plant from west Bengal named as Aloe

barbadensis showed quite different constitutions. The principal component of the gel was a pectic substances containing mainly galacturonic acid and was accompanied by lesser amounts of a galactan, arabinan and a non acetylated glucomannan (Table – 6 and 7).

A.vera plant from south India into four partially acetylated glucomannans, the whole having an average glucose/mannose ratio of 1:6, although the individual ratio varied from 1.5:1 to 1:19. The molecules were linear with 1-4 linkages between the sugars units. Traces of galactouronic acid, galactose, xylose and arabinose were also found [9]. Recent study of *A.vera* analysis important factor neglected in many analyses is the effect of seasonal and cultivar variation. "None of the studies took in to consideration seasonal, climatic and soil variations which may strongly affect the compositional of the gel". These factors could explain the different results obtained by different experimental workers. The healing agent of may not be present in the leaves at all times but is found there only during certain season of the year.[24]

CONCLUSION

The present study revelaed the role of sspecific plant compounds such as anthroquinones and dihydroxy anthroquinones as well as saponins have been proposed to have direct antimicrobial activity. It may be considered as a fruitful approach towards the search of new drug. The overall results of the antimicrobial and photochemical activity of the leaf and gel extracts of *Aloe vera* justified the traditional uses of plant and suggested that the indigenous traditional medicines could be used as a guide in the continuing search of new natural products with potential medicinal properties.

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Source of support: Nil, Conflict of interest: None Declared