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Separation and Characterization of the Phenolic Anthraquinones from *Aloe Vera*

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Abstract: The experiment was carried out at Department of Inorganic Chemistry, University of Madras, Tamilnadu, India, to separate and characterize the phenolic anthraquinones from *Aloe vera*. Among the different forms of *A.vera*, A.vera sap contains more phenolic anthraquinones of 7.09 mg/g of lyophilizate than the remaining forms *viz.,* aloe skin and aloe gel. All the three forms of aloe and the chromotographed fraction of aloe sap in five different solvent mixtures were characterized by infrared spectroscopy, fluorescence spectroscopy, mass spectroscopy and U.V. spectroscopy. Aloe sap fractions were all characterized by fluorescent spectroscopy and their emission wavelengths were comparable to that of phenolic anthraquinones.

Keywords: *Aloe vera*, phenolic anthraquinones, separation, characterization

INTRODUCTION

Aloe vera L. (*Aloe barbadensis* Miller) is an important medicinal plant belongs to the family Liliaceae. It has larger demands and is traded in medicinal drug markets of the world for flavouring liquid and a source of 'aloin' (4.5 to 25 per cent). In recent times, herbal remedies are gaining their prominence, because of the observation that the efficacy of allopathic medicines such as antibiotics, which once had near universal effectiveness against serious infections is on the wane. Over the years, infectious agents have developed resistance to synthetic drugs and the herbs and their active constituents are now being increasingly used to treat various diseases. The ability of herbal medicine to affect body systems depends on the chemical constituents that it contains. Aloe products have long been used in health foods and for medical and cosmetic purposes. These products range from aloe drink to aloe gels, powders, capsules, creams etc. for both internal and external uses for a wide variety of indications. Aloe has a wide range of medicinal application such as wound healing effect, reduces blood sugar in diabetes, soothes burns, eases intestinal problems, reduces arthritic swelling, ulcer curative effect, stimulates immunes response against cancer etc. Anthraquinones derivatives in *A. vera* gel play an important role in the treatment of tumors, diabetes, ulcer and cancer[2]. Keeping this fact in view, the present study was undertaken to isolate the phenolic anthroquinones from the methnolic extract from *A. vera* leaf gel.

MATERIALS AND METHODS

The study was carried out at Department of Inorganic Chemistry, University of Madras, Guindy campus, Tamilnadu, India, to separate and characterize the phenolic anthraquinones from the *A.vera* sap. The modified methodology has been adapted for the separation of phenolic anthraquinones. The phenolic anthraquinone derivatives are separated from aloe gel, aloe skin and aloe sap by TLC, HPLC and column chromatography. The methonal extract was prepared by taking 10g. of aloe sap in 500 mL of methanol and stirred for 4-6 hr and the methanolic extract was filtered. The extract was subjected to rotary evaporation under vacuum at room temperature. The extraction was repeated for 5-6 times. The powdered aloe sap was subjected to TLC and HPLC to find out the various fractions in it. Then the fractions were separated by a modified elution technique. The same process was repeated for aloe skin and aloe gel. Elution technique was done by packing the brown coloured A. *vera* sap powder in a column by using 120-mesh column chromatography silica gel with hexane. Then the column was eluted in succession with ethyl acetate (1.5 l), ethyl acetate/acetone (4:1, v/v; 1 l), ethyl acetate/acetone (3:1, v/v; 1 l), acetone (1 l), and methanol (1.5 l). Each elute was separately

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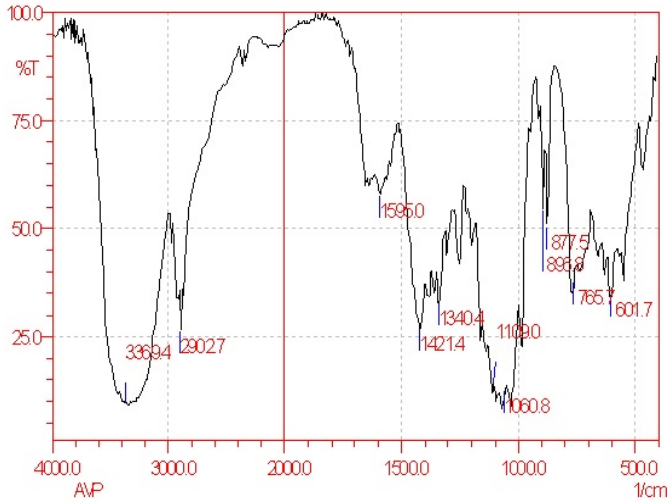


Fig. 1: I.R. Spectrum of Aloe Sap

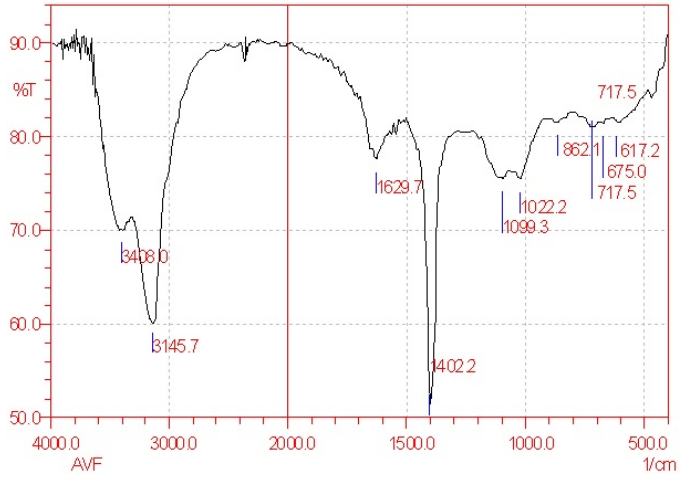


Fig. 2: I.R. Spectrum of Aloe Gel

Fig. 3: I.R. Spectrum of Aloe Skin

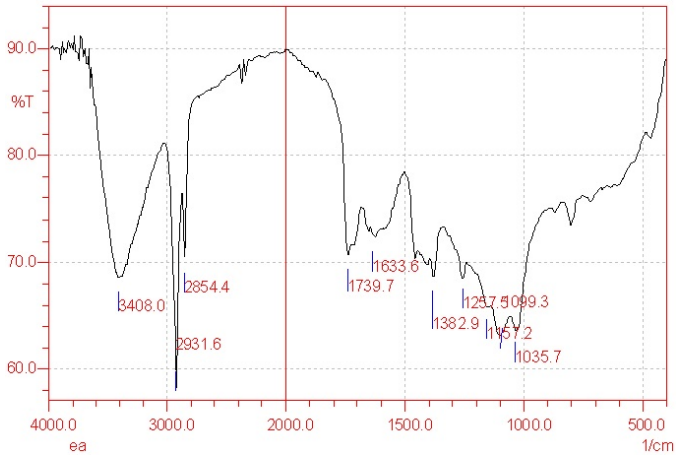


Fig. 4: I.R. Spectrum of Ethyl Acetate Fraction

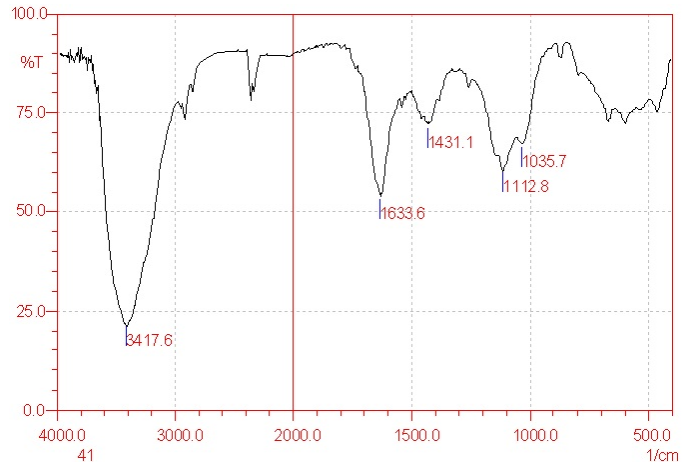


Fig. 5: I.R. Spectrum of Ethyl Acetate/Acetone (4:1) Fraction

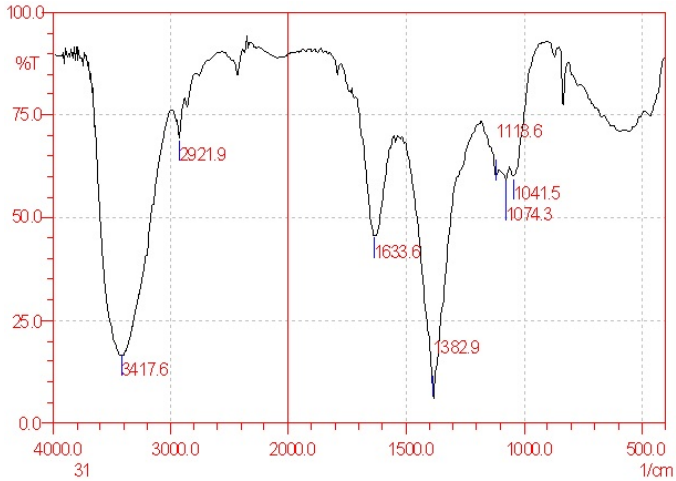


Fig. 6: I.R. Spectrum of Ethyl Acetate/Acetone (3:1) Fraction

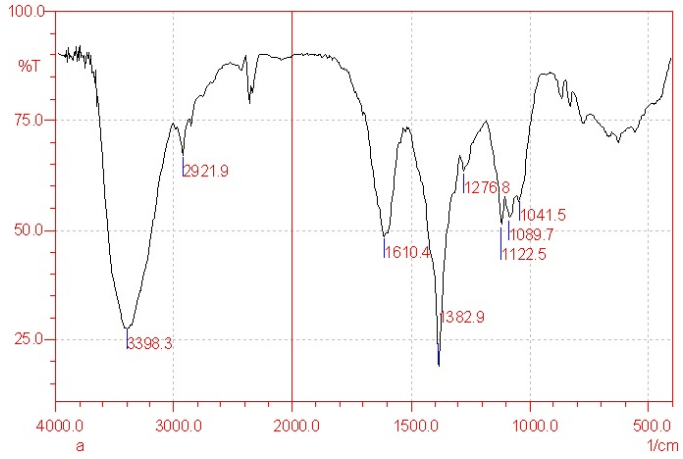


Fig. 7: I.R. Spectrum of Acetone Fraction

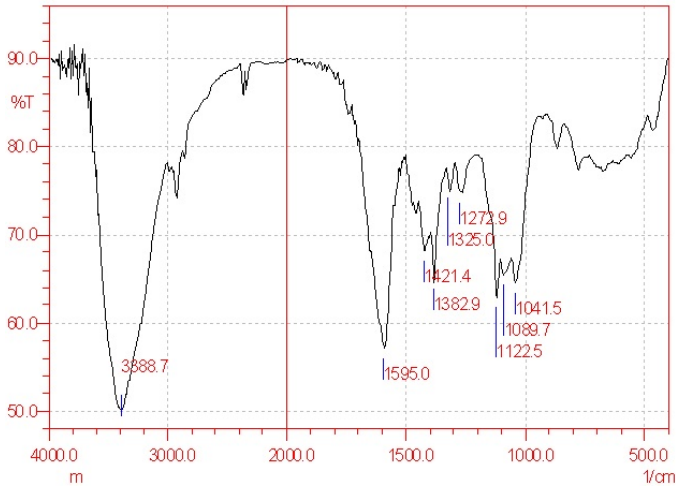


Fig. 8: I.R. Spectrum of Methanol Fraction

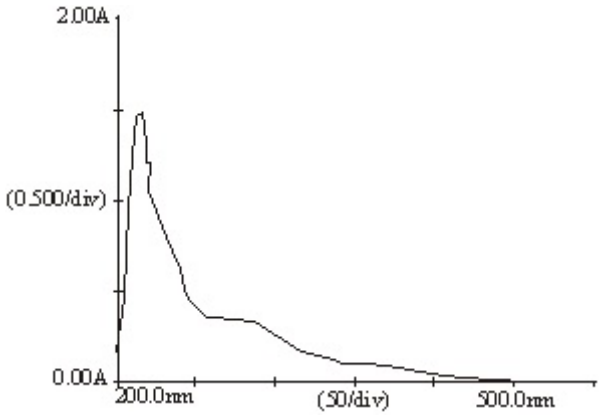
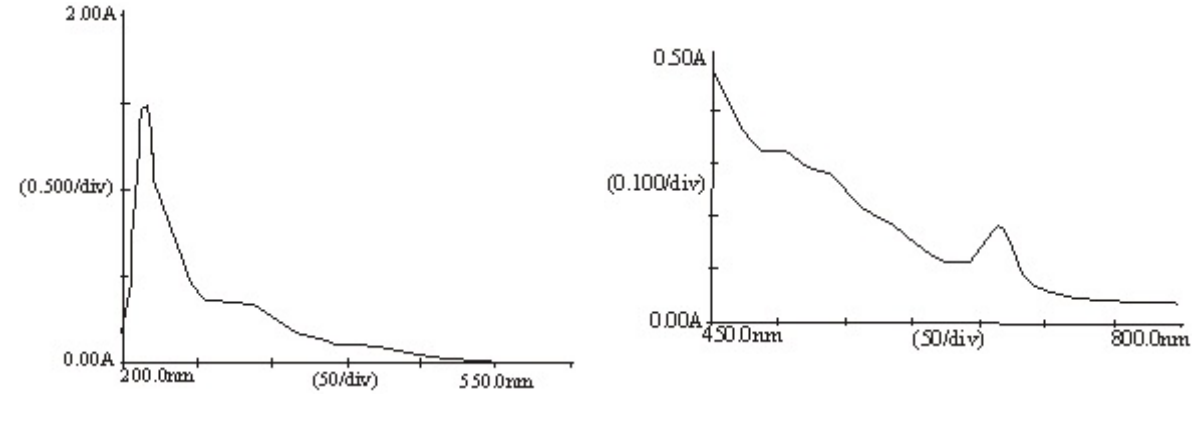
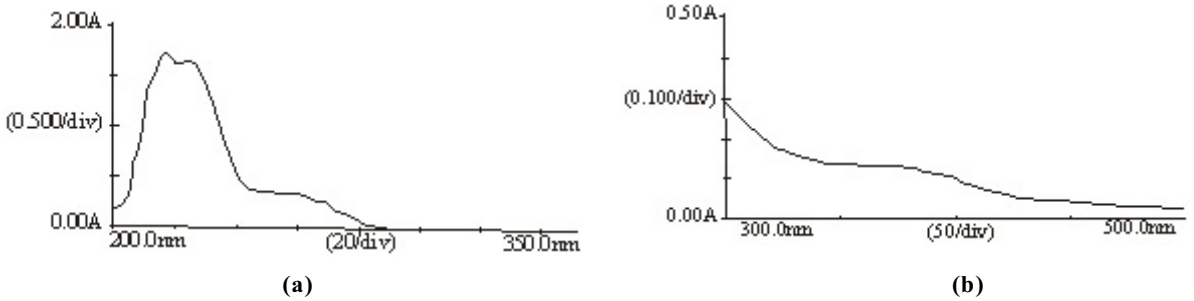


Fig. 9: Electronic spectra of Aloe Sap.



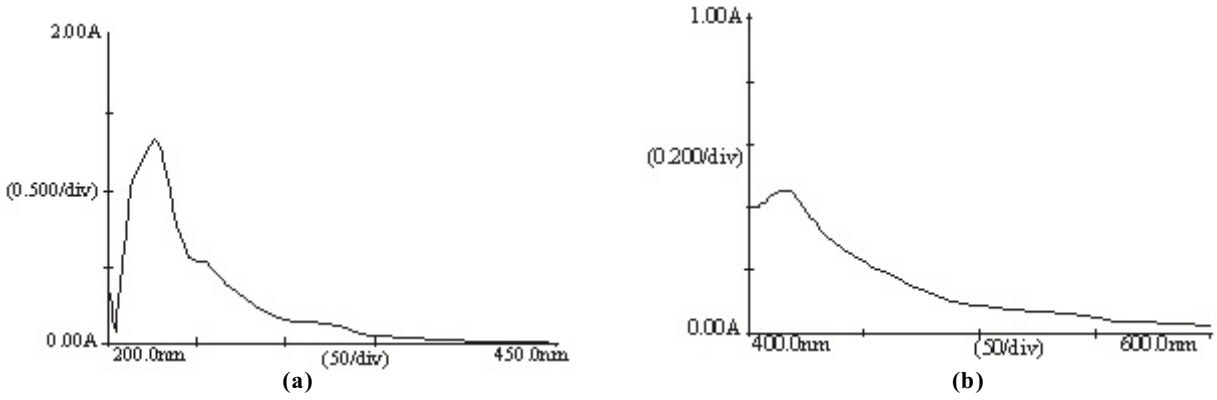
(a) **(b)**

Fig. 10: Electronic spectra of Aloe Skin.



(a) **(b)**

Fig. 11: Electronic spectra of Aloe gel.



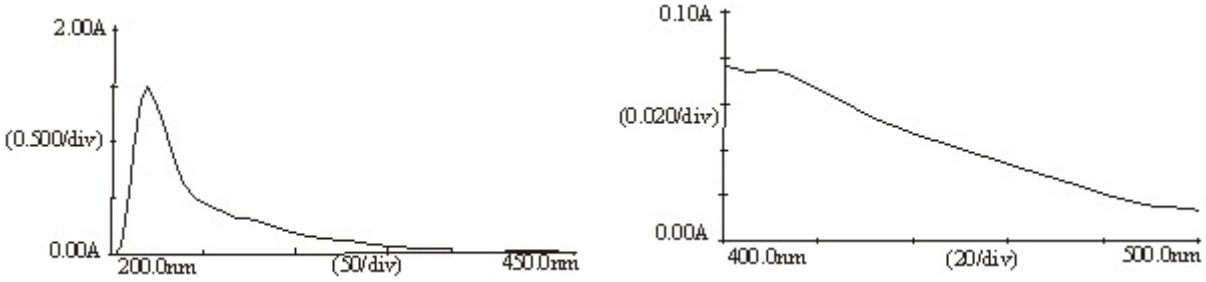
(a) **(b)**

Fig. 12: Electronic spectra of Ethyl acetate fraction of Aloe Sap extract.

evaporated to dryness in vacuo and dissolved in 5 ml of methanol before storage at -20˚C. The presence of phenolic anthraquinones was confirmed by using the modified Borntragers test and the bromine test. The modified Borntragers test was done by adding 100 mg. of aloe sample with 10 ml of $FeCl_3$ and 5 ml of HCl. The mixture was heated and filtered. After the mixture was cooled, 10 ml of CCl_4 was added and CCl_4 layer was separated. It was washed with 5 ml of water and 5 ml of ammonia solution. The Bromine Test was done by adding 2 ml of aloe filtrate to 2 ml of freshly prepared bromine water. The presence of phenolic anthraquinones can be found out by changing of the colour of the solution from rose pink to cherry red. The separated Phenolic anthraquinones were characterized by using Infrared spectroscopy, UV-Visible spectroscopy, Fluorescence spectroscopy, Mass spectroscopy and elemental analysis.

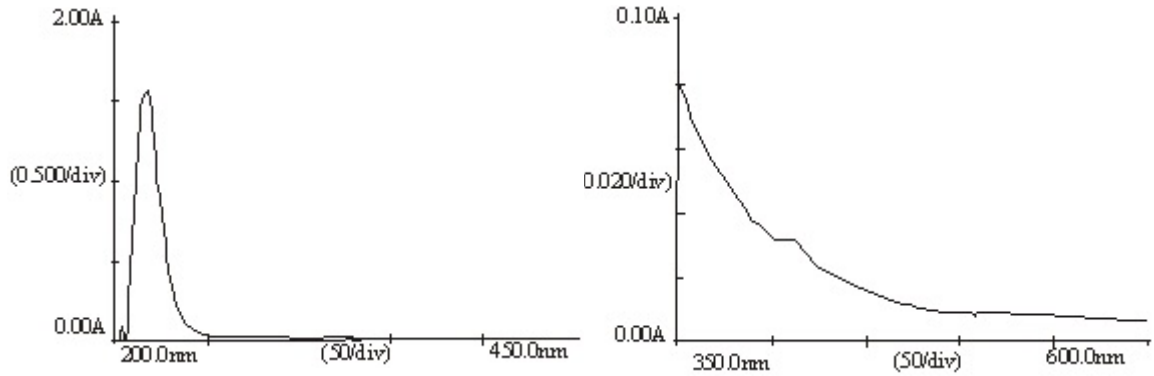
Table 1: Phenolic Content of Subfractions of Methanolic Extracts of *Aloe vera* sap

Fraction	Total phenols (mg/g lyophilizate)			
Solvent system	Volume (l)	Aloe Sap	Aloe Skin	Aloe Gel
Ethyl acetate	1.5	2.35	0.63	1.87
Ethyl acetate/acetone (4:1)	1.0	0.82	0.21	0.39
Ethyl acetate/acetone (3:1)	1.0	0.37	0.24	0.35
Acetone	1.0	0.92	0.37	0.75
Methanol	1.5	2.63	0.59	1.46
Total Phenolic content (mg/g)		7.09	2.04	4.82



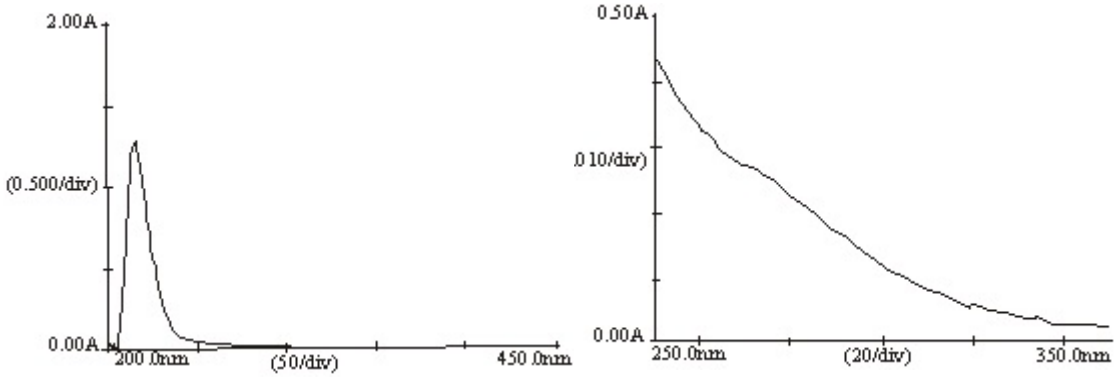
(a) **(b)**

Fig. 13: Electronic spectra of Ethyl acetate/Acetone (4:1) fraction of Aloe Sap extract.



(a) **(b)**

Fig. 14: Electronic spectra of Ethyle acetate/Acetone (3:1) fraction of Aloe Sap extract.

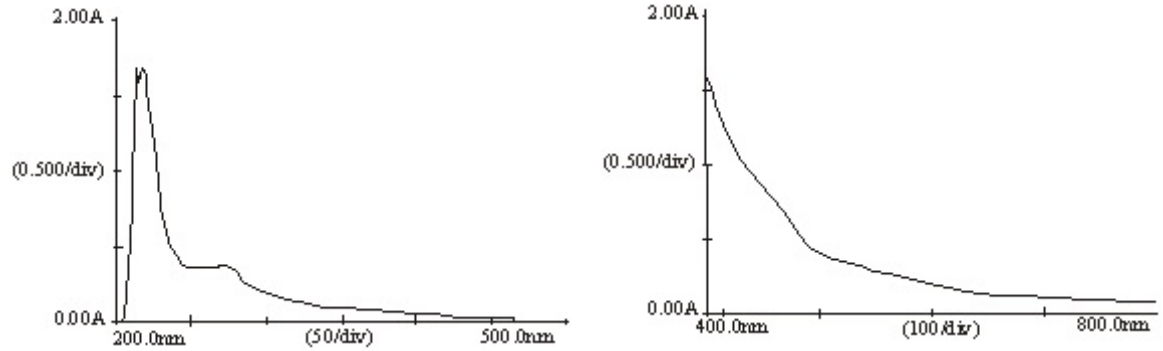


(a) **(b)**

Fig. 15: Electronic spectra of Acotone fraction of Aloe Sap extract.

Table 2: Infra- red spectral data for Aloe vera compounds

Functional group	Infra-red spectral data Wave length (1/c.m)			Infra-red spectral data for A. vera sap sub-fractions Wave length (1/c.m)				
	Aloe sap	Aloe skin	Aloe gel	Ethyl acetate	Ethyl acetate/ Acetone(4:1)	Ethyl acetate/ Acetone(3:1)	Acetone	Methanol
Phenolic-OH	3369.4	3398.3	3408.0	3408.0	3417.6	3417.6	3398.3	3388.7
		3155.3	3145.7					
C=O	1600.0	1623.9	1629.7	1633.6	1633.6	1633.6	1610.4	1595.0



(a) **(b)**

Fig. 16: Electronic spectra of Methol fraction of Aloe Sap extract.

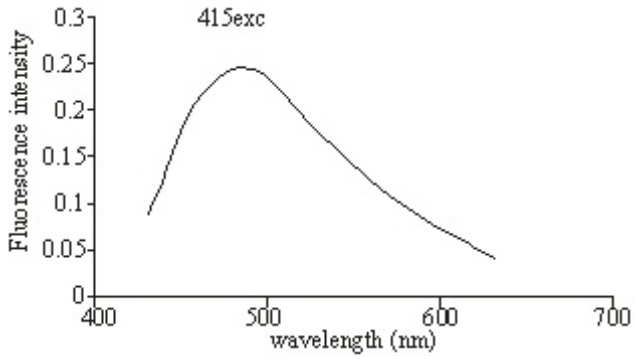


Fig. 17: Fluorescence Spectrum of Ethyl Acetate Fraction

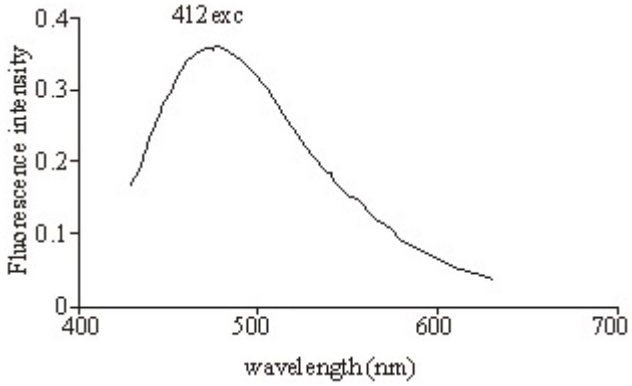


Fig. 18: Fluorescence Spectrum of Ethyl Acetate/Acetone (4:1) Fraction

Table 3: Electronic spectral data for Aloe vera compounds

	Electronic spectral data Wave length (1/c.m)			Electronic spectral data for A. vera sap sub-fractions Wave length (1/c.m)				
	Aloe sap	Aloe skin	Aloe gel	Ethyl acetate	Ethyl acetate/ Acetone(4:1)	Ethyl acetate/ Acetone(3:1)	Acetone	Methanol
Absorbance (λmax)	220, 267, 366	219, 270	217, 268, 360	223, 265, 336, 415	217, 270, 335, 412	219, 325, 410	217, 275, 407	219, 272, 415

Table 4: Fluorescence spectral data for Aloe sap sub-fraction

Compounds	Ethyl acetate	Ethyl acetate/Acetone (4:1)	Ethyl acetate/Acetone (3:1)	Acetone	Methanol
Emission wave length (λem, nm)	484	479	467	475	490

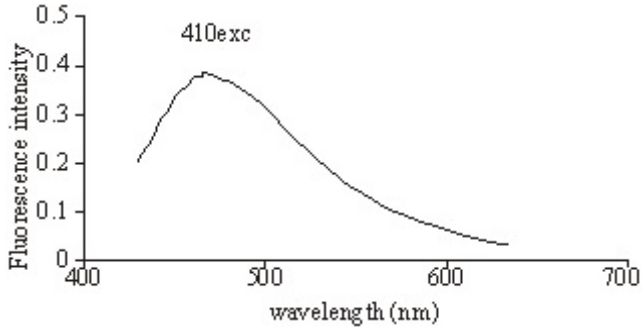


Fig. 19: Fluorescence Spectrum of Ethyl Acetate/Acetone (3:1) Fraction

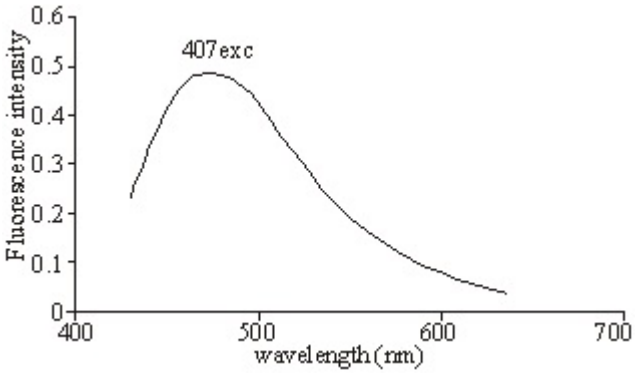


Fig. 20: Fluorescence Spectrum of Acetone Fraction

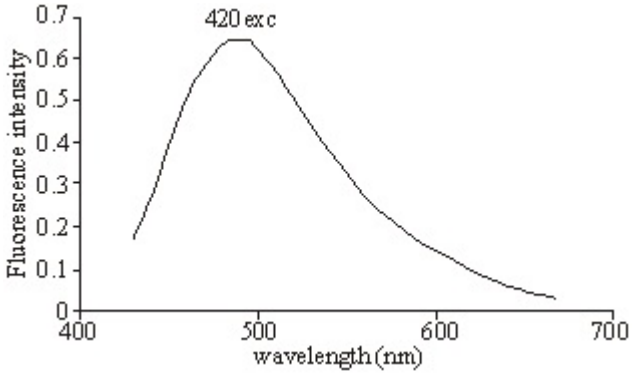


Fig. 21: Fluorescence Spectrum of Methanol Fraction

RESULTS AND DISCUSSIONS

Among the different forms of *A. vera,* aloe sap contains more phenolic anthroquinones than the remaining forms viz., aloe skin and aloe gel. The colour of the solution was changed from rose pink to cherry red indicating the presence of phenolic anthraquinones. The phenolic content of subfractions of methanolic extracts of A. *vera* is tabulated in Table1. Among the different forms of the *A.vera,* Aloe sap contains more phenolic anthraquinones of 7.09 mg/g lyophilizate. All the three forms of aloe (aloe sap, aloe skin and aloe gel) (Fig.1-3) and the chromatographed fractions of the aloe sap in five different solvent mixtures were characterized by infrared spectroscopy (Fig.4-8). Characteristic HPLC profiles of fresh and aged aloe solution detected at 360 and 220 nm. Several anthroquinones were separated and identified[4]. HPLC analysis of the anthroquinone content showed that all the samples contained significantly less than that of the raw, unwashed aloe gel[3]. The peak values of the functional groups are shown in Table 2. *A. vera* sap, A. *vera* skin, A. *vera* gel (Fig.9-11) and the chromatographed fractions of Aloe gel were all characterized by U.V.spectroscopy (Fig.12-16) are shown in Table 3. The peaks around 220nm, 270nm and 366nm are the characteristic peaks for phenolic anthraquinones. Aloe sap fractions were all characterized by fluorescence spectroscopy (Fig.17-21) and their emission wavelengths were comparable to that of phenolic anthraquinones. The spectral data are shown in Table-4. Aloe sap contains phenolic anthraquinones was already reported by[1].

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