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A Comparative study on Antioxidant, Proximate analysis, Antimicrobial activity and phytochemical analysis of *Aloe vera* and *Cissus quadrangularis* by GC-MS

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ABSTRACT

Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history it has been used as a popular folk medicine. *Aloe vera* and *Cissus quadrangularis* these are used as medicinal values, *Aloe vera* contains both medicine and cosmetic effect. Screening of phytochemical (Qualitative and Quantitative) analysis of *Aloe vera* and *Cissus quadrangularis* shows that almost of the chemical constituents are present Tannin, Phlobatannins, Saponin, Flavonoids, Steroids, Terpenoids, and Cardiac glycosides Anthroquinones which are used in medicinal purpose. Both Aloe vera and *Cissus quadrangularis* are having the antimicrobial activity against human pathogens. In 100% concentration of extraction zone of inhibition is high. But 25%, 50% and 75% shows the lowest inhibition activity. Proximate analysis indicates the nutrients efficacy. In GC-MS analysis some of the Phytocomponents are screened as Squalene Oleic Acid, Dodecanoic acid in *Aloe vera*. Eugenol, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid, diisooctyl ester, Phenol, 2,4-bis(1-phenylethyl)- are present in *Cissus quadrangularis*.

Key words:*Aloe vera, Cissus Quadrangularis*, Antioxidant activity, proximate analysis, Antimicrobial activity and phytochemical analysis.

INTRODUCTION

Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 1996). *Aloe vera and Cissus quadrangularis* are more responsive to nutrient, grows in arid climates and widely distributed in India and other arid areas. Both species are used in folk medicine. *Aloe vera* extracts may be useful for numerous medical and cosmetic applications since ancient times (Morton, 1961). *Aloe vera* contains over nutrients and 200 active compounds, including vitamin, enzyme, minerals, sugars, lignin, Anthraquinones, Saponins, Slcilylicacid and amino acids (Park and Jo, 2006). Numerous scientific studies on *Aloe vera* demonstrating its analgesic, anti-inflammatory wound healing immune modulating and anti-tumor activities as well as anti-viral, anti-bacterial and anti-fungal properties (Anonymous, 2008).

Phenolic compounds are the second major substances found in *Aloe vera*. Aloesin has been reported to have a skin whitening effect (Yagi, 1977). The main active constituent of *Aloe vera* plant extract is aloine, an anthraquinone heteroside (Bruneton, 1993). The fresh stem and leave of *Cissus quadrangularis* is used for the treatment of hemorrhoid, menstrual disorder, scurvy and as anti-flatulence. In India it is used for many diseases (Chopra et al., 1958; Yoganarsimhan, 2000). Phytochemical studies of *Cissus quadrangularis* found several phytochemical constituents such as flavonoids, Triterpenoids (Bhutani et al., 1984; Gupta andVerma, 1990; Mehta et al., 2001), Murthy et al. (2003) reported the antibacterial and antioxidant activities of the extract from *Cissus quadrangularis*.

MATERIALS AND METHODS

Collection of plant materials

Fresh, Healthy and non infected *Aloe Vera* and *Cissus quadrangularis* stem were collected in Thanjavur. Collected aloe vera was washed with distilled water and kept it in room temperature for air dried. Dried aloe vera was powdered and kept it in polythene bags for further uses.

Sample Preparation

Aqueous extract of *Aloe Vera* and *Cissus quadrangularis* samples were used to carry out the Qualitative and Quantitative analysis using standard procedures to identify the phyto constituents as described by Sofowara (1993) Trease and Evans (1989) and Harbone (1973).

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Phytochemical Screening

Test for Tannins (Mac, 1963)

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black colouration.

Test for Phlobatannins (Iyenger. 1995)

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

Test for Saponin (Ramakrishnan, 1994)

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids (Iyenger. 1995)

5ml of the diluted ammonia solution was added to a portion of aqueous filterate of plant extract followed by the addition of concentrated sulphuric acid formation of yellow color.

Test for Steroids

Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H2S04. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Terpenoids (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Cardiac glycosides (Keller-Killani test)

2 ml of glacial acetic acid containing one drop of ferric chloride solution was add to 5ml of the aloe vera extract. This was sunder layer with 1ml of concentrated sulphuric acid. Formation of a brown ring at the interface indicates the presence of cardiac glycosides.

Test for Anthroquinones

0.5gm of the extract was boiling with 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette out into another test tube & 1ml of diluted ammonia was added. The resulting solution was observed for colour change.

Proximate Analysis
Proximate analysis for *Aloe Vera* and *Cissus quadrangularis* i.e., Moisture, Protein, Fat, Fiber, Ash and Insoluble Ash.

Quantitative Analysis
Quantitative Analysis of *Aloe Vera* and *Cissus quadrangularis was* carried out by described method as Alkaloid (Harbone, 1973), Saponin (Obadoni and Ochuko, 2001), Phenols (Malic et al., 1980), Flavanoid, Tannin (Robert 1971). Results are shown in Table 3.

Antioxidant Activity
Aloe Vera and *Cissus quadrangularis* air dried powdered samples (10g) were extracted with 250ml of methanol using the soxhlet extractor for 72 hrs at a temperature not exceeding the boiling point of the solvent. From the extracted sample antioxidant activities was carried out by DPPH Method (Lin et al., 1999) and FRAP Method (Benzie, F.F and Strain, J.J.1999). Results are shown in Table 4.

Antimicrobial Activity
Aloe Vera and *Cissus quadrangularis* extracts were prepared in different concentration of 25%, 50%, 75%, 100% was tested against two bacterial and two fungal strains such as *Pseudomaonas solanacearum and Xanthomonas citri, Aspergillus niger, Aspergillus oryzae*, All the cultures were obtained in pure form from the culture collection of Institute of Microbial Technology (IMTECH), Chandigarh, India. Respectively these organisms were compared in the two different media like nutrient agar media for bacteria, potato dextrose agar media for fungi and Mueller Hinton agar media for comparative studies. The Minimum Inhibitory concentration (MIC) values are determined and observed for presence or absence of visible inhibition for respective plates. Results are shown in Table 5.

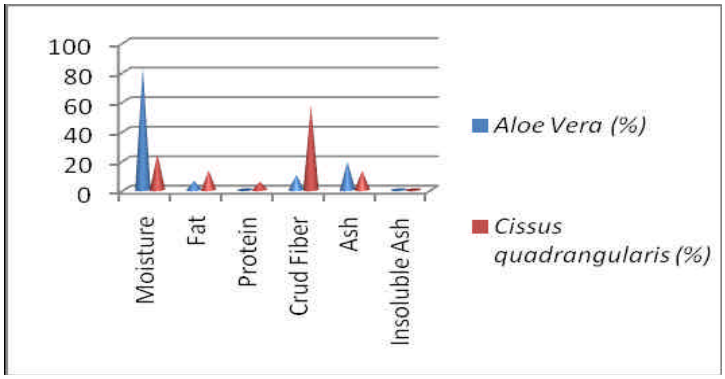
RESULTS AND DISCUSSION
Aloe Vera and *Cissus quadrangularis* shows that the Tannin, Saponin, Flavonoids, Anthroquinones are present while Phlobatannins, Steroids and Cardiac glycosides are absent. Terpenoids present in *Aloe vera* but absent in *Cissus quadrangularis* (Table.1). Phytochemical studies of *Cissus quadrangularis* found several phytochemical constituents such as flavonoids, Triterpenoids (Bhutani *et al*., 1984; Gupta and Verma, 1990; Mehta *et al*., 2001),

Qualitative analysis of the Phytochemicals in *Aloe Vera and Cissus quadrangularis* (Table.1).

S.No	Parameter	*Aloe Vera*	*Cissus quadrangularis*
1.	Tannin	Presence	Presence
2.	Phlobatannins	Absence	Absence
3.	Saponin	Presence	Presence
4.	Flavonoids	Presence	Presence
5.	Steroids	Absence	Absence
6.	Terpenoids	Absence	Presence
7.	Cardiac glycosides	Absence	Absence
8.	Anthroquinones	Presence	Presence

Proximate Analysis
In proximate analysis Aloe vera shows that high moisture, and low fat and protein, while Cissus quadrangularis showed the high fat, protein and fiber. (Fig. 1)

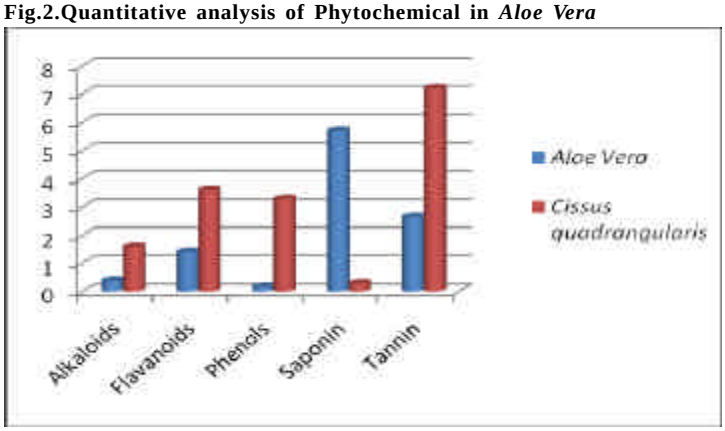
Fig. 1. Proximate Analysis of *Aloe Vera and Cissus quadrangularis*



Quantitative analysis of Phytochemical in *Aloe Vera and Cissus quadrangularis*

In *Cissus quadrangularis* are the best Phytocomponents than *Aloe vera*, but the saponin content is high in *Aloe vera*. Alkaloid, Flavanoids, Phenols and Tannin are highly present in Cissus Quadrangularis.Phenolic compounds are the second major substances found in *Aloe vera*. Aloesin has been reported to have a skin whitening effect (Yagi, 1977). (Fig. 2)

Fig.2.Quantitative analysis of Phytochemical in *Aloe Vera*

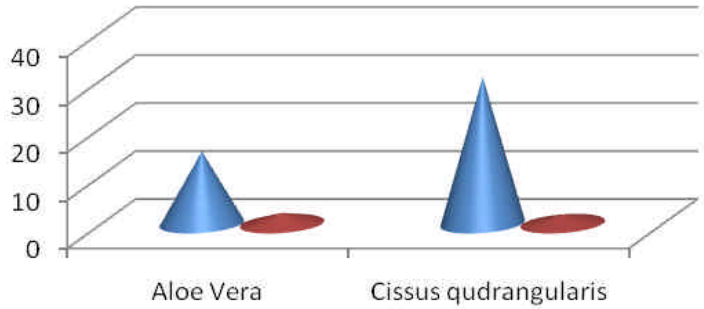


Determination of Antioxidant activity *Aloe Vera and Cissus quadrangularis*
Murthy et al. (2003) reported the antibacterial and antioxidant activities of the extract from *Cissus quadrangularis*. Antioxidant activity is high in *Cissus Qudrangularis* about 30.60% while the Aloe vera activity is 15.08 % in DPPH Method. Table. 2 and (Fig. 3)

Table. 2.Antioxidant activity by DPPH and FRAP Method.

S.No	Test for Antioxidant activity	*Aloe Vera*	*Cissus qudrangularis*
1.	DPPH Method (Inhibition %)	15.08	30.608
2.	FRAP Method (mM/100g)	2.28	1.96

Fig.3. Graphical representation of Antioxidant activity by DPPH and FRAP Method.



Antimicrobial activity
Numerous scientific studies on *Aloe vera* demonstrating its analgesic, anti-inflammatory wound healing immune modulating and anti-tumor activities as well as anti-viral, anti-bacterial and anti-fungal properties (Anonymous, 2008).

Table 3: Antimicrobial activity of different concentrations of *Aloe Vera and Cissus quadrangularis*

Pathogenic Organisms	Diameter of Inhibition Zone in mm against various concentrations of Herbal Extract							
	Aloe vera (mm)				*Cissus quandrangularis (mm)*			
	25 %	50 %	75 %	100 %	25 %	50 %	75 %	100 %
Xanthomonas compestris	14	16.2	18.1	21.3	12.1	15.2	16.8	22
Pseudomonas fulva	18	20	21	25	08	11	13	17
Aspergillus niger	10	14	16	19	13	17	18.5	21
Aspergillus flavus	14	17	20	22	15	15	17	20

Aloe vera and *Cissus Quandrangularis* are shows that 100% concentration of extracts perform maximum zone of inhibition in both Bacterial (*Xanthomonas compestris* (21.3mm) and *Pseudomonas fulva* (25 mm) and fungal (*Aspergillus niger* (19 mm) and *Aspergillus flavus* (22 mm) activity (Table: 3).

GC Analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30mm×0.25mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 El was employed (split ratio of10:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. (Fig 4&5)

Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. Table 4 & 5.

Fig.4. GC-MS studies of *Aloe vera*-036

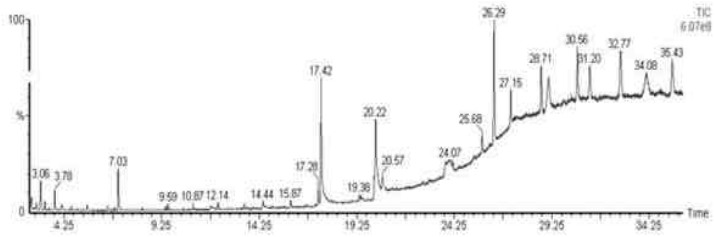


Table: 4.Components identified in *Aloe vera*

No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	3.06	p-Xylene	C_8H_{10}	106	4.88
2.	3.78	Cyclohexane, nitro-	$C_6H_{11}NO_2$	129	2.67
3.	4.13	Decane	$C_{10}H_{22}$	142	1.19
4.	4.63	Limonene	$C_{10}H_{16}$	136	0.44
5.	5.44	Undecane	$C_{11}H_{24}$	156	0.70
6.	7.03	1-Heptanol, 2-propyl-	$C_{10}H_{22}O$	158	5.86
7.	9.47	7-Tetradecene, (E)-	$C_{14}H_{28}$	196	0.33
8.	9.59	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_{30}$	198	0.49
9.	10.87	Hexadecane	$C_{16}H_{34}$	226	0.56
10.	11.77	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.37
11.	12.14	Nonadecane	$C_{19}H_{40}$	268	0.66
12.	13.49	Eicosane	$C_{20}H_{42}$	282	0.62
13.	14.44	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.60
14.	15.87	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	1.41
15.	17.42	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	27.63
16.	19.38	9,12-Octadecadienoic acid, methyl ester, (E,E)	$C_{19}H_{34}O_2$	294	1.07
17.	19.47	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	1.03
18.	20.22	Oleic Acid	$C_{18}H_{34}O_2$	282	22.26
19.	27.15	Eicosane	$C_{20}H_{42}$	282	5.18
20.	28.71	Heptacosane	$C_{27}H_{56}$	380	9.94
21	31.20	Squalene	$C_{30}H_{50}$	410	10.12

CONCLUSION

According to the results *Aloe vera and Cissus quadrangularis* shows that presence of Tannin, Saponin, Flavonoids, Anthroquinones. Terpenoids present in *Cissus quadrangularis.* Proximate analysis indicates that Nutrients are in both *Aloe vera and Cissus quadrangularis. Moisture and Ash are high in Aloe vera*. While *Cissus quadrangularis* presents more fat, protein, and Crude fiber than *Aloe vera*. In quantitative analysis of Phytochemical content *Cissus Quadrangularis* shows the maximum amount of Phytocomponents saponin content is high in Aloe vera. Antioxidant activity was high in *Cissus quadrangularis in DPPH Method in FRAP Method it shows the lowest activity than the Aloe vera. Cissus quadrangularis* (Ethanolic Extract) shows the highest zone of inhibition in *Pseudomonas solanacearum.* When 25% of *Cissus quadrangularis* (Ethanolic Extract) shows the highest zone of inhibition in *Aspergillus niger. Cissus quadrangularis* Ethanolic Extracts used to screen all phytocomponents by using Gas Chromatography method. According to the results some of the medicinal value components are present in the plant extracts such as Asarone, Phytol, Phenol, 2,4-bis(1-phenylethyl)- which are all have medicinal properties. Squalene is used in cosmetics as a natural moisturizer. Oleic acid is used as an emulsifying or solubilizing agent in aerosol products. Stearic acid is useful as an ingredient in making candles, plastics, dietary supplements, oil pastels and cosmetics, and for softening rubber. The essential fatty acids are all omega-3 and -6 methylene-interrupted fatty acids. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K_1. It is used along with simple sugar or corn syrup as a hardener in candies.

Fig.5. GC-MS studies of *Cissus quadrangularis*

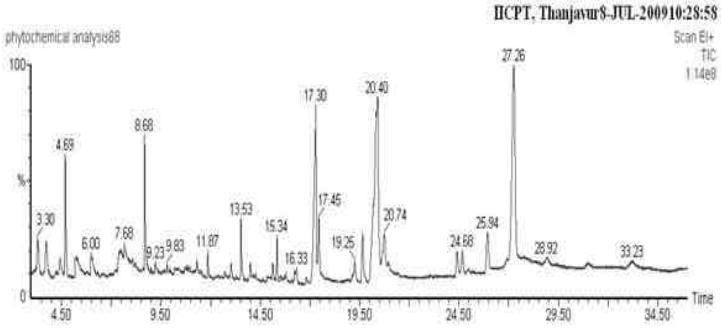


Table: 5.Components identified in *Cissus quadrangularis*

S.No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	4.69	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	4.23
2.	8.68	Eugenol	$C_{10}H_{12}O_2$	164	4.92
3.	11.32	Undecanoic acid	$C_{11}H_{22}O_2$	186	0.87
4.	11.87	Asarone	$C_{12}H_{16}O_3$	208	0.78
5.	13.53	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	$C_{15}H_{18}$	198	2.33
6.	14.00	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.91
7.	15.13	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.65
8.	15.34	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	1.31
9.	16.33	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	0.60
10.	17.30	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.59
11.	17.45	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	4.38
12.	19.65	Phytol	$C_{20}H_{40}O$	296	2.93
13.	20.40	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	27.17
14.	20.74	Octadecanoic acid	$C_{18}H_{36}O_2$	284	5.52
15.	24.41	Methanone, [1,4-dimethyl-7 -(1-methylethyl)-2-azulenyl]phenyl-	$C_{22}H_{22}O$	302	1.36
16.	25.94	Phenol, 2,4-bis(1-phenylethyl)-	$C_{22}H_{22}O$	302	3.03
17.	27.26	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	26.41

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REFERENCE

1. Mace, M.E., "Histochemical localization of phenols in healthy and diseased tormto roots, phytopathology,, 1963, 14:915-925.
2. Iyenger,M.H., "Study of ocrude drug" 1995, 8:2
3. Ramakrishnan, S., Prasannan, K.G., and Rajan, R.," Text book of medical biochemistry" 1994, Orient Longman, New Delhi, India.
4. Obadoni, B.O., Ochuko,P.O., "Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic platns in Edo and Delta states of Nigereia. Global J.Pur Appli. 2001, Sci-8:203-208.
5. Malic, C.P., Singh M.B., "In plant enzymology and histo enzymology ad kalyani publisher, New delhi, 1980. P.286.
6. Robert, E.B., Agro, J., 1971, 63, P-511.
7. Benzie, F.F., Strain, J.J., "Ferric reducing/Antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration methods in enzymology-1999, 299:15-23.
8. Trease, G.E., Evans, W.C.,". Pharmacognosy", 11th edn. Brailliar Tiridel can, Macmillian publishers. 1989

9. Sofowara, A., Medicinal plants and Traditional medicine is Africa spectrum Books LTD., Ibadan, Nigeria .1993, P.289.
10. Harbone, J.B., "Phytochemical method, London Chapman and Hall, Ltd., 1973. 49-188.
11. Bruneton, J., "Pharmacognosie", Phtochimie Plantes Medicinales. Tec.Doc,Paris, 1993 PP.363.
12. Park, Young, In Jo, Tae Hyung, "Perspective of Industrial application of Aloe vera"., New Perspectives on Aloe., 2006, PP 191-200.
13. Anonymous, Aloe vera:History, Sciencee and Medicinal uses, http://www/healing aloe.com ,2008.
14. Yagi, A., Harad, N., Yamada, H., Iwadare, S., Nishioka, I., " Antibrady Kinin active material in Aloe saponaria". J pharm Sci .1982,71 (10): 1172-4.
15. Morton JF "Folk uses and commercial exploitation of *Aloe* leaf pulp". *Econ. Bot* 1961,15:311–319.
16. Boudreau MD, Beland FA "An evaluation of the biological and toxicological properties of Aloe barbadensis (miller), Aloe vera". *Journal of environmental science and health. Part C, Environmental carcinogenesis & ecotoxicology reviews* , April 2006, **24** (1): 103–54. doi:10.1080/10590500600614303. PMID 16690538
17. King GK, Yates KM, Greenlee PG, *et al.* "The effect of Acemannan Immunostimulant in combination with surgery and radiation therapy on spontaneous canine and feline fibrosarcomas". *Journal of the American Animal Hospital Association* 1995, **31** (5): 439–47. PMID 8542364
18. Eshun K, He Q "Aloe vera: a valuable ingredient for the food, pharmaceutical and cosmetic industries—a review". *Critical reviews in food science and nutrition* 2004, 44 (2): 91–6. Doi:10.1080/10408690490424694. PMID 15116756

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