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Phytochemical and XRD analysis of *Aloe vera* leaf powder

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Abstract

The aim of the study was to investigate the phytochemical compounds present in Aloe vera leaf extract with petroleum ether, ethanol and chloroform as solvents. The petroleum ether extract showed the presence of alkaloids, flavanoids, steroids, terpenoids, anthraquinone, protein, quinone, carbohydrate, tannin, and saponin. The ethanol extract showed the presence of alkaloids, flavanoids, proteins, phenol, carbohydrate and tannin. Similarly chloroform extract showed the presence of alkaloids, flavanoids, steroids, terpenoids, protein, phenol, quinone and saponin.

Key-Words: *Cosmetics, Phytochemicals, X-ray diffraction, Terpenoids*

Introduction

Aloe vera is a succulent plant species that store water in their leaves, stems, and roots to prepare for times of little or no rain. Its thick leaves contain the water supply for the plant to survive long periods of drought. (Foster, 1999). It is native to North Africa and the Caribbean. It is found only in cultivation, having no naturally occurring populations. This nontoxic plant can survive in hot temperatures ranging all the way to 104 degrees Fahrenheit and down to below freezing temperatures as long as the root of the plant is not damaged. Its use has been documented for thousands of years. *Aloe vera* leaf has a unique look with no stem and has spear like spikes which is protected by serrated edges. The leaf is made of a gel, which is colourless, viscous liquid consisting primarily of water and polysaccharides and has a bitter taste (Brinelson, 1995). Over 250 species of the genus *Aloe* are existing, with only two species grown on commercial basis (*Aloe barbadensis* and *Aloe arborescens*).

plant because of its various healing properties. Today, Ayurvedic, Hoemoeo and Unani physicians utilize numerous species of medicinal plants that found their way a long time ago into the Hindu Material Media (Narayana Rao and Thammanna K, 1987). 6000 years old stone carvings in Egypt contain images of the plant, which they referred to as the "plant of immortality" was given as a burial gift to deceased pharaohs. This magical plant is used for the treatment of various kinds of diseases and illness since ancient time that's why *Aloe vera*, is also known as the 'plant of immortality'.

Numerous scientific studies on *A. vera* are demonstrating its analgesic, anti-inflammatory, wound healing, immune modulating and anti-tumor activities as well as antiviral, anti-bacterial, and antifungal properties (K. Fujita, *et al.*, 1978). Two types of exudates are secreted by aloe leaves. One is a bitter reddish-yellow juice contained in the pericyclic cells located under the strongly cutinized epidermis of the leaves. This "juice" has been generally used for laxative purposes and in dried form. Its bitterness is due to the presence of aloin, aloe-emodin and related compounds. The other exudate is a transparent, slippery mucilage or gel produced by the thin-walled tubular cells in the inner central zone (parenchyma) of the leaf. The raw "gel" resembles colorless gelatin with hair-like connective matrices and is also sometimes called "juice." In antiquity, this mucilage was applied to inflamed skin and during the 20th century it was used on radiation burns.

The bio active compounds are used as astringent, haemostatic, antidiabetic (Yongchaiyudha S, *et al.*, 1996) and (Bunyapraphatsara N, *et al.*, 1996), antiulcer, anti-septic (Hirat and Suga T, 1983) antibacterial (Rabe, T. and J. Van Staden, 1997) anti-inflammatory, antioxidant and anticancer agent and also, effective in treating stomach ailments, gastrointestinal problems, skin diseases, constipation, radiation injury, wound healing, burns, dysentery, diarrhoea and in the treatment of skin diseases. Currently the plant is widely used in skin care, cosmetics and as nutraceuticals (Lorenzetti, *et al.*, 1964). Herbal medications gain popularity due to a perception that there is a lower incidence of adverse affect in plant preparation compound to synthetic

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Pharmaceuticals. According to the World Health Organization, medicinal plants would be the best source for obtaining a variety of drugs (Santos *et al.*, 1995). In the present study we focused on finding out the presence of some of the phytochemicals present in Aloe vera leaf.

Material and Methods

Preparation of leaf powder

Aloe vera leaves were obtained from the Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu. Leaves that were much closer to the ground alone were selected as they are more mature. These leaves were washed with distilled water to remove any soil or dirt sticking on its outer surface. Using a sharp knife the thorny edges of the leaves were removed. The leaves were cut length wise, the slimy mucilaginous gel and the clear inner gel were removed carefully using a butter knife. The thick green outer rind alone was retained, washed again with distilled water and air dried under shade for 2 to 3 weeks. Dried leaves were powdered using an electric pulverizer. Fine powder was obtained by sieving (Figure – 1).

10 g of the leaf powder was weighed using an electronic balance (Denver XS-210) and made into packets using Zerohaze filter paper (A Grade, SD's). These powders were subjected to extraction with 500 ml of the solvents for 8h using a Soxhlet apparatus (Harbourne, 1973 and Vogel, 1978). Petroleum ether (60 – 80°C) extraction was followed by chloroform and ethanol extraction. The powders were subjected to extraction with solvents in the order of increasing polarity. The leaf extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40°C. The residue thus obtained was stored in tightly closed glass vials in the refrigerator for further use.

Phytochemical screening

Qualitative analysis

Preliminary phytochemical screening of the lemon juice extract was carried out using the standard procedures of Raman N. (2006). The extracts were tested for alkaloids, tannins, phenols, flavonoids, sterols, saponins, anthraquinones, proteins, quinines, and carbohydrate.

Particle characterization

The X-ray diffraction (XRD) patterns of the samples were recorded on a PANalytical X'Pert PRO X-ray diffractometer using Cu K α radiation ($\lambda = 0.15406 \text{ \AA}$). The crystallite size of nanocrystalline samples was measured

from the line broadening analyses using Debye-Scherrer formula after accounting for instrumental broadening

(Equation 1):

$$D_{XRD} = 0.89 \lambda / \beta \cos \theta \dots\dots\dots(1)$$

Where λ – wavelength of X-ray radiation used in \AA , θ is the diffraction angle, β is the full width at half maximum (FWHM) in radians in the 2θ scale, D_{XRD} is the crystallite size in nm.²¹



Fig. 1: Aloe vera

Results and Discussion

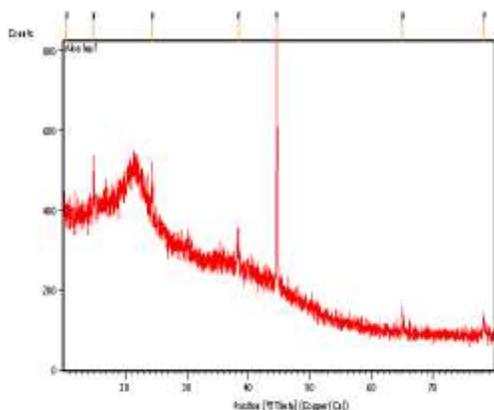
Bioactive compounds such as alkaloids, flavonoids, steroids, terpenoids, anthraquinone, proteins, phenols, quinone, carbohydrates, tannins and saponins were detected to be present in the leaves of aloe vera. Since this plant had been used in the treatment of different ailments such as malaria, dysentery, diarrhea, skin burn etc, the medicinal roles of these plants could be related to such identified bioactive compounds. The presence of these biologically active compounds in the extracts has made the plant to be known for its medicinal use especially for antimicrobial activity against pathogenic organisms (Table.1). A. vera contains amino acids, lipids, sterols, tannins, enzymes, chromones (flavonoids) and mannose-6-phosphate (Brinelon, 1995; Davis and Robson, 1999; Santrel International, 1998). This plant had been found useful in the treatment of wound, burns, skin disorders and anti-inflammatory activity (McCouley, 1990; Shellon, 1996; Davis and Robson, 1999). Aloe contains Campesterol, F2 Sitosterol and Lupeol (Coats, 1979). It is an aspirin like compound present in Aloe plant possessing anti-inflammatory and anti-bacterial properties. Topically, it

has a ketolytic effect which helps to debride a wound of necrotic tissue.

Table 1 Phytochemical analysis of Aloe vera leaf powder

S.No	TEST	PET ETHER	ETHANOL	CHOLOROFORM	
1	Alkaloids	i)Mayer	+	-	-
		ii)Wagner	-	+	+
		iii)Hagers	-	+	-
2	Flavanoids	i)Sod.Hydroxide test	-	+	-
		ii)Sulphuric acid test	+	+	+
3	Steroids	Libermann-Burchard	+	-	+
4	Terpenoids	Libermann-Burchard	+	-	+
5	Anthraquinone	Borntragers	+	-	-
6	Protein	i)Ninhydrin (Aq)	+	+	-
		ii)Ninhydrin (Acetone)	-	-	-
		iii)Biuret	-	-	+
7	Phenols	i)Ferric Chloride	-	-	-
		ii)Libermann	-	+	+
8	Quinone	Conc HCl test	+	-	+
9	Carbohydrate	i) Molish	-	+	-
		ii)Fehlings A &B	+	+	+
10	Tannin	ii)Acetic acid	+	+	-
		Dil HNO3	-	+	-
11	Saponin	Foam Test	+	-	+

Peak list of XRD



Pos.[°2Th.] d-	Height [cts]	FWHMLe ft[°2Th.]	spac[Å]	Rel.Int. [%]
10.5273\	10.83	0.8029	8.40362	2.06
14.8771	95.47	0.2007	5.95489	18.19
24.3929	102.23	0.1004	3.64916	19.48
38.3805	77.35	0.2007	2.34537	14.74
44.5883	524.83	0.1004	2.03220	100.00
65.0232	46.63	0.2676	1.43438	8.88
78.2512	29.18	0.4015	1.22173	5.56

Fig. 2: XRD analysis of Aloe vera leaf powder

XRD can be used to characterize the crystallinity of nanoparticles and it gives the average diameters of all the nanoparticles. The fine particles were characterized by XRD for structural determination and estimation of crystallite size. XRD pattern were analyzed. All experimental peaks were matched with theoretically generated one and indexed. The XRD patterns of all the samples were shown in Figure 2.

Tannin has been reported to interfere with bacterial cell protein synthesis and is important in the treatment of ulcerated or inflamed tissues and also in the treatment of intestinal disorders (Igbnosa, *et al.*, 2009). Alkaloid has also been reported to be a pain killer and saponin has managing effect against inflammation (Igbnosa *et al.*, 2009; Hussain *et al.*, 2009). Flavonoid is also important against inflammation and microorganisms. Saponins which are soapy substances forming 3 per cent of the gel are general cleansers, having antiseptic and anticarcinogen properties (Hirat and Tsuga 1983). Minerals found in *A. vera* are calcium, zinc, chromium, potassium. Magnesium lactate inhibits histidine decarboxylase and prevents the formation of histamine from the amino acid histidine (M. S. Shelton, 1991). *Aloe* plant contains 25 percent of solid fraction that contain sugars. Sugar acts as immune modulators capable of enhancing and retarding the immune response (Green 1996; Kahlon *et al.*, 1991; Sheets, 1991). Concentrated extracts of *Aloe* leaves are used as laxative and as a haemorrhoid treatment.

Vitamins namely A, B1, B2, B6, B12, C and E, which the human body cannot prepare by itself, are available in *A. vera*. Vitamin B complex and C are to play an important role in reducing stress and inflammation (Coats, 1979). *Aloe* contains the enzymes such as amylase, lipase and carboxypeptidase. Lipase can digest by breaking down fats and sugars. Amylase hydrolyzes starch to liberate dextrin. The activity of serum amylase is increased in acute pancreatitis. The pancreatic carboxypeptidase is metalloenzymes. It inactivates bradykinin and produces an anti-inflammatory effect. During the inflammatory process, bradykinin produces pain associated with vasodilation and its hydrolysis to produce an analgesic effect (Obata, 1993), (Shelton, 1991). Anthroquinone is a phenolic compound found in the sap. These compounds exert a powerful purgative effect, which are potent antimicrobial agents and possess powerful analgesic effects (Lorenzetti, *et al.*, 1964), (Sims *et al.*, 1971).

A. vera gel provides 20 of the 22 necessary amino acids required by the human body. Minerals are defined as natural components formed through

geological processes needed in small amounts to regulate body functions.

Conclusion

Since *Aloe vera* plants had been used in the treatment of different ailment such as malaria, dysentery, diarrhea, skin burn etc, the medicinal roles of these plants could be related to such identified bioactive compounds. Efforts should be geared up at characterizing the entire bioactive agents present, in the two plants for their full utilization.

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