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Comparative study of morphology and nutritional properties of bitter and non bitter genotype of *Aloe vera*

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Abstract
Aloe is used for several medicinal purposes therapeutic applications and antioxidant properties. *Aloe vera* contains large number of bioactive active compounds. These plants are one of the richest sources of health for human beings coming from nature. The purpose of this study is the investigation of morphology and nutritional difference between to *Aloe vera* genotypes. The differences of functional groups were studied by mean of Fourier Transformed Infrared Spectroscopy (FTIR). Bitter type *Aloe vera* gel shows higher amount of Saponin and flavanoids than non bitter *Aloe vera*. Total phenolic content was also higher in bitter *Aloe vera* gel. Structural properties show the Smoothness and uniformity in the surface topography of bitter and non bitter *Aloe vera*.

Keywords: *Aloe vera*, Phytocheicals, Saponins, Phenols, SEM, FTIR

Introduction
Aloe vera is well known for their nutraceutical and cosmoeceutical properties. *Aloe vera* is widely used as a natural treatment and alternative therapy for various diseases. Aloe leaf gel contains over 75 nutrients and 200 active compounds including 20 minerals, 18 amino acids and 12 vitamins. *Aloe vera* provides 7 out of the 8 essential amino acids which the body cannot synthesis (Chang *et al.* 2006; Pisalkar *et al.* 2011) [5, 13]. Aloe is rich in all vitamins excluding Vitamin D, especially the antioxidant Vitamins A (beta-carotene), C and E and traces of Vitamin B_{12}. Calcium, sodium, potassium, manganese, magnesium, copper, zinc, chromium and the anti-oxidant selenium are also present in *Aloe vera* (Coats, 1979) [6]. Sugars are derived from the mucilage layer of the plant which surrounds the inner gel and are known as muco polysaccharides, which enhance the immune system and help to detoxify the unwanted components. Twelve phenolic compounds are found exclusively in the plant sap. Saponins form about 3 per cent of the gel. The importance of plants has been established and well documented by scholars since ancient period. Apart from number of social benefits, much emphasis has been accorded to the plants of medicinal value. In this study, we determined and compared the phytochemical contents and antioxidant capacities of *Aloe vera* lyophilized leaf gel. This was done not only to describe *Aloe vera* leaf gel genotype with regard to phytochemical contents and possible health benefits but also to compare the different genotypes present.

Material and Methods
Material
Mature *Aloe vera* leaves were harvested from the Botanical garden of Department of Medicinal and Aromatic Plants, University of Horticulture and Forestry, H.P. The harvested leaves were thoroughly washed with tap water and weighed. The side, base and tip of the leaves were then, removed and the inner portion were cut longitudinally into strips. The gel parenchyma was then cut away from the rind. The inner leaf gel was freeze dried and vacuum packed for further analysis.

Physicochemical Analysis
Moisture, protein, lipid, fiber and ash were determined with standard procedures (AACC, 2000)[1]. Titratable acidity was estimated by titration method using phenolphthalein as an indicator (AOAC, 1990) [2]. The total sugars were determined according to the method of Sadasivam and Manickam (1992) [16].

Phytochemical analysis

The flavonoid content in the sample of *Aloe vera* was determined by the method given by Boham and Kocipai-Abyazam (1974) [3]. Sample was extracted repeatedly with 100 ml of 80 per cent aqueous methanol at room temperature. The whole solution was filtered. The filtrate was later transferred in to a crucible and evaporated to dryness over a water bath and weighed. Total phenolic content of the extracted *Aloe vera* sample was determined by the method given by Singleton and Rossi (1965) [17]. Sample solution was prepared in ethanol. A 100 µl of sample extract was diluted with 5 ml of distilled water was mixed with 0.5 ml of Folin–Ciocalteu reagent. After 10 minutes, 1.5 ml of 20 per cent solution of sodium carbonate was added, and content was mixed properly. Prepared samples were kept for 1 h at room temperature, and the absorbance was measured at 765 nm.

Antioxidant Activity (DPPH radical scavenging assay)

The assay was conducted according to the procedure described by Ozsoy *et al*. (2008) [11]. Freshly prepared methanolic solution of DPPH (3.9ml, 6x 10-5M) was added to sample. α- Tocopherol (0.1-10 mg/ml) was used as positive control. After 45 min. of incubation the absorbance was recorded at 517 nm. The measurements were made in triplicate and averaged.

Fourier Transforms Infrared (FTIR) spectrophotometric analysis

The FTIR analysis was carried out following KBr pasting method given by Vazquez *et al.* (2008) [18], by using Perkin Elmer RX1 Spectrophotometer (Germany). The KBr Pellets contain 2 mg of freeze dried *Aloe vera* sample. The spectrum was recorded within 4000-450 cm^{-1} wave number region.

Microstructure of *Aloe vera*

The morphology of the powders of mucilage was evaluated using a EmCraft (Korea): Table-top scanning electron microscope (SEM Cube-1000). Samples were dehydrated by putting them into critical point drying equipment. The mucilage powder was fixed in an aluminum plate, using an electrically conductive tap and a coating of gold at 10 mbar for 90 s was applied. The microscope was operated at 5 kV and different levels of magnification: 500X, 1000X, 1500X, secondary electron mode.

Stastical Analysis

All experiments were performed in triplicates. All values are expressed as mean ± standard deviation (SD) of three separate experiments.

Result and Discussion

Physical properties

This experiment was conducted to study the phenotypic variations among two selected genotypes i.e. bitter and non bitter. Ten randomly selected mature plants of each genotype were chosen for recording observations and mean data used for statistical analysis. The table 1 shows the mean values and standard derivations of the composition results obtained for the *Aloe vera gel*. The non bitter *Aloe vera* leaf had higher leaf length, however bitter *Aloe vera* shows higher gel weight. Gel yield is an important character in determining suitability to food or cosmetic industry. According to Paez et al. (2000) [12], an increase in leaf thickness of Aloe plants with moisture corresponds with increase in gel production. It was observed that both genotypes differed in colour of leaves showing dark green in non bitter as compared to yellowish green colour leaves in bitter genotypes. Gel was relatively firm in bitter *Aloe vera* leaves with firmness reading 3.375 kg and 2.900 kg for non bitter. The firmness is important, especially for hand filleting because gel cannot be effectively removed if the leaves are too flaccid (O'Brien *et al.* 2011)[10].

Refractive index is the physical property of gel to determine the purity of gel as compared to double distilled water. Gel with lowest refractive index is the best for extraction process. More refractive index indicates the impurities in the extracted gel (Chandegara and Varshney, 2014) [4]. There was significant difference between bitter and non bitter *Aloe vera* for refractive index.

Nutritional properties of *Aloe vera* gel

Determination of moisture contents is one of the most fundamental and important analytical procedure. The non bitter *Aloe vera* gel had higher moisture content, since moisture content of *Aloe vera* was very high, the other components appear to occur in very small concentration. The crude protein content of *Aloe vera* genotypes varied significantly and 1.901 per cent for bitter type and 0.880 per cent for non bitter *Aloe vera*. Similarly crude fat, ash percentage, crude fiber, total carbohydrates and acidity (% mallic acid) was high in bitter type *Aloe vera* gel. Carbohydrates are important in foods as a major source of energy, to impart crucial textural properties and as dietary fiber which influences physiological processes. Non-digestible polysaccharides (all those other than starch) comprise the major portion of dietary fiber (Nielsen SS, 2009) [9].

Phytochemical Properties

The soluble protein of *Aloe vera* gel was 1.453 mg/100gm and 0.548 mg/100gm for bitter and non bitter *Aloe vera* gel respectively. Both genotypes were statistically found different from each other. The values for total sugars were 0.767 and 0.568 per cent for bitter and non bitter *Aloe vera* gel respectively. Total sugars were higher in bitter type *Aloe vera* gel.

Total phenolic content of the plants was measured using the Folin-Ciocalteu method and the results are presented in fig 1. There was a wide range of phenol concentrations in the medicinal plants as the values varied from 56.310 mg/100gm bitter *Aloe vera* gel and 38.353 mg/100gm for non bitter aloe. Vega-Galvez *et al.* (2011) [19] reported 96.81 mg GA/100 dm total phenols in *Aloe vera*. Flavonoids represent a large family of low molecular weight phenolics. They show antioxidant activity. The flavonoid content of bitter *Aloe vera* gel was 35.089 per cent and non bitter contain 28.159 per cent. Vidic *et al.* (2014) [20] reported the flavonoid content of peel gel extract and of commercial *Aloe vera* sample. They observed highest flavonoid in peel extract (9.17±0.19 mg (QE)/g); while the lowest was in gel extract (0.29±0.03 mg (QE)/g). It has been observed that values for saponin varied significantly from 31.080 per cent for bitter and 25.609 per cent for non bitter respectively.

The antioxidant activity of *Aloe vera* was determined using DPPH method. The reduction capability of DPPH radical was determined by the decrease in absorbance at 517 nm induced by the antioxidants. Fig 1 illustrates a variation in DPPH scavenging activity of the *Aloe vera* genotypes which was 41.314% for bitter and non bitter *Aloe vera* had minimum DPPH scavenging activity (32.804%).

FTIR

The IR spectra of bitter (Fig. 2) and non bitter (Fig. 3) *Aloe vera* gel were basically indistinguishable in the wave-number range of 4000–400 cm^{-1}. Different polar groups such as –OH (corresponds to 3417.35 and 3401.18 cm^{-1}), CH_2 (corresponds to 2917.58 and 2934.06 cm^{-1}), =CO (corresponds to 1626.90 and 1629.33 cm^{-1}), COO− (corresponds to 1423.89 and 1421.15 cm^{-1}), −COC (corresponds to 1033.28 and 1042.91 cm^{-1}) appeared in the FTIR spectrum of *Aloe vera* gel. The identification of the functional groups implies the presence of specific compounds, and functional group detection also helps in the physico-chemical characterization of any material of interest with reference to pertained biochemical and biological activities (Ray *et al.,* 2013; Ray and Gupta, 2013) [15, 14].

Phenolic −OH stretching frequency appeared with strong and broad intensity of bands at around 3417.58 and 3401.18 cm^{-1} for both genotypes. CO= stretching at 1626.90 33 cm^{-1} appeared with strong intensity in bitter and with weak intensity in non bitter at 1629.33 cm^{-1} and broad shaped bands indicating the presence of carbonyl compounds (Femenia *et al.,* 2003) [7]. Weak absorption peaks in between 656.75.62 cm^{-1} in bitter *Aloe vera* might be associated with C-H bending indicating the presence of polymerized carbohydrates and the phenolics. The IR response around 1626.90 cm^{-1}and1033.28 cm^{-1}were more pronounced in bitter *Aloe vera* gel, imply the higher acetylation in the same (Femenia *et al.,* 2003; Ray and Gupta, 2013) [7, 14].

Structural Characterization

The Scanning Electron Microscopy (SEM) was carried out for conspicuous visualization of freeze dried *Aloe vera* gel. The SME was conducted at magnification of 500x and 1500x. The figure 4(a, b) and 5(a,b) shows the morphological and microstructure difference observed between two *Aloe vera* genotypes. Images for bitter and non bitter *Aloe vera* gel reveals that freeze dried gel was amorphous, varied from smooth to undulating terrain, and resembled broken glass and flake like structures. The amorphous polydispersed structure accounts for freeze drying mediated sublimation of water molecules from the parenchymal *Aloe vera* gel matrix. Loizou *et al.* (2006) [8] reported that a high degree of inter-molecular cross-linking reduces the number and the size of the pores in rehydrated gel film. Smoothness and uniformity in the surface topography of both bitter and non bitter accounts for better cross-linking of structural polysaccharides. SEM analysis, the measures of water holding potential and polysaccharide content estimation affirm that the degree of cross linking among the component polysaccharides of Aloe gel is decisively influenced by the qualitative and quantitative attributes of component polysaccharides and with the growth periods.

Table 1: Physical properties of Bitter and Non Bitter *Aloe vera* gel

Parameters	Bitter	Non Bitter	CD (P≤0.05)
Leaf Length (cm)	66.215±0.995	76.550±0.656	2.581
Leaf Width (cm)	7.550±0.104	8.900±0.188	0.465
Gel Weight (gm)	603.763±3.124	514.000±1.927	7.949
Peel Weight (gm)	422.036±13.58	478.149±1.403	29.566
Specific Gravity	1.006±0	1.001±0	0.001
Firmness (kg)	3.375±0.025	2.900±0.027	0.079
RI	1.335±0.001	1.333±0	0.002

Table 2: Nutritional properties of Bitter and Non Bitter *Aloe vera* gel

Parameters	Bitter	Non Bitter	CD (P≤0.05)
Moisture (%)	96.313±0.044	97.721±0.01	0.172
Crude Protein (%)	1.901±0.017	0.880±0.001	0.039
Crude Fat (%)	0.503±0.009	0.445±0.007	0.024
Ash (%)	0.413±0.008	0.341±0.014	0.034
Crude Fiber (%)	0.767±0.005	0.568±0.006	0.016
Total Carbohydrates (%)	1.052±0.001	1.008±0.001	0.002
Acidity (% Malic acid)	0.060±0.067	0.050±0	0.001
Soluble Protein (mg/gm)	1.453±0.026	0.548±0.017	0.067
Total Sugars (mg/gm)	0.767	0.568	0.016

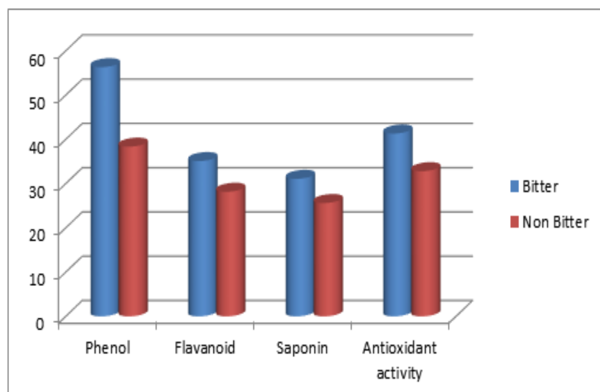

Fig 1: Functional properties of *Aloe vera* genotype

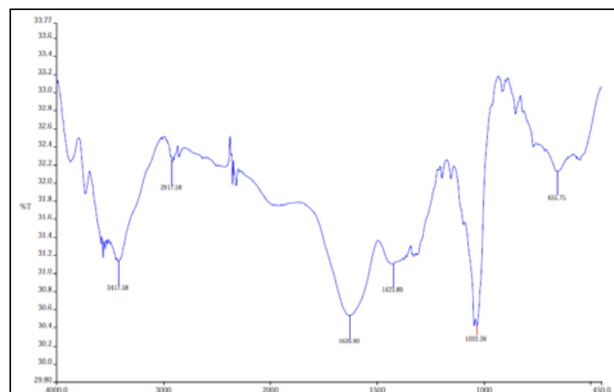

Fig 2: FTIR spectra of Bitter *Aloe vera* gel

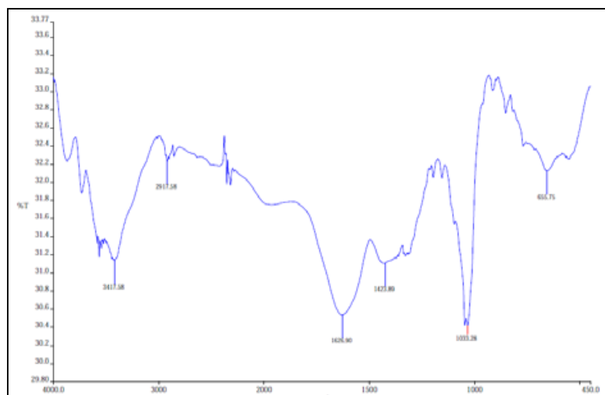

Fig 3: FTIR spectra of Bitter *Aloe vera* gel

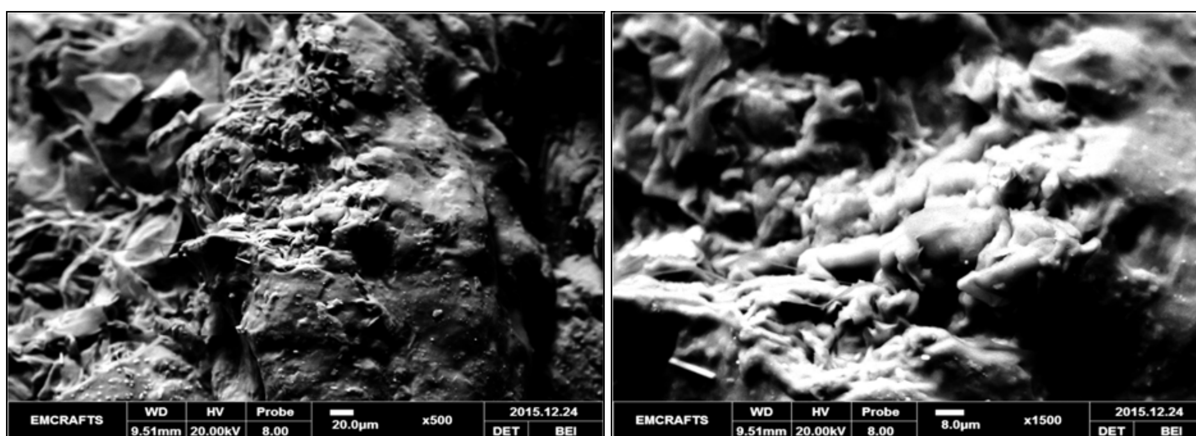

Fig 5: Cryo-SEM micrograph of Bitter *Aloe vera* gel (a) X 500 (b) X 1500

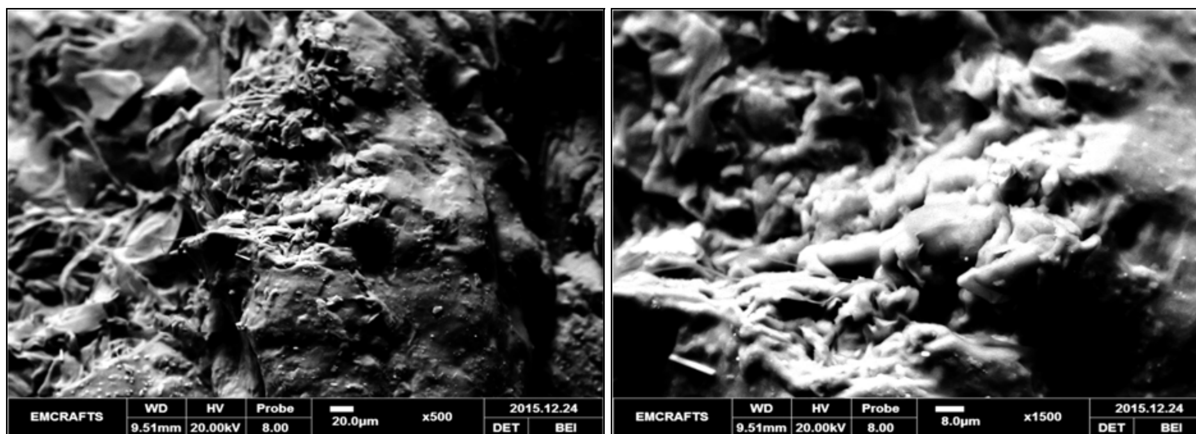

Fig 6: Cryo-SEM micrograph of non Bitter *Aloe vera* gel (a) X 500 (b) X 1500

Conclusion

There are numbers of secondary metabolites found in plants which contribute significant biological activities. Phytochemical analysis of plants is commercially important being great interest of pharmaceutical and food industries. This study indicated that the both bitter and non bitter genotype contain a number of nutrients and bitter *Aloe vera* genotype had higher gel per cent then non bitter *Aloe vera*. Phytocompouds viz. phenols, flavanoids and saponins were more in bitter genotype. It can be concluded that bitter *Aloe vera* had higher nutritional value than non bitter genotype. Structural studies show the smoothness and uniformity in the surface topography of both bitter and non bitter accounts for better cross-linking of structural polysaccharides.

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