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PHYTOCHEMICAL EVALUATION OF DIFFERENT *ALOE SPECIES*

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ABSTRACT

Plants claim the largest source of phytochemical constituents which take part in relieving many health problems. So a screening of phytochemical constituents was performed in the laboratory in selected Aloe species.Qualitative and quantitative determinations of phytochemical constituents were evaluated in Aloe species namelyAloe barbadensis, Aloe rupestris, Aloe juvenna and Gasteria pulchra. Phytochemical screening results revealed the presence of almost all the biologically active secondary metabolites like alkaloids, cardiac glycosides, flavanoids, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids in the Aloe extracts.But steroids were absent in Aloe juvenna and cardiac gycosides were absent in Gasteria pulchra. Quantitative analysis of these compounds by spectrophotometry revealed that all the Aloe species possessed significant phytochemical constituents with slight variation. Aloe barbadensis possessed maximum alkaloids (23.83 ± 0.28), flavanoids (18.66 ± 0.57), phenolics (14.26 ± 0.25), tannins (4.53 ± 0.3) and terpenoids (13.5 ± 0.86) than other species except for saponins. Thus the present study justified that Aloe species contain medicinally important bioactive compounds that it is nutritionally as well as medicinally valuedin pharmaceutical field.

Keywords *– Alkaloids, Flavanoids, Phenolic compounds, Steroids, Tannins, Terpenoids.*

1. INTRODUCTION

Plants have been traditionally used worldwide extensively as medicines due to free of side effects. Plants are the richest source of biological compounds that is perceived in medicines, nutraceuticals, food supplements, pharmaceutical intermediates, folk medicines and in synthetic drugs[1]. A medicinal herb is a multitude of phytochemical compounds like alkaloids, flavanoids, steroids, glycosides, saponins, resins, oleoresins, terpenoids, anthraquinones, reducing sugars, essential and non-essential aminoacids, proteins, phenols, lactones and essential oils.These phytochemicals serves the plants to develop defense mechanism against predators like microorganisms, insects, pesticides and herbivores[2]. So researchers are looking forward to explore new potent drugs from plants by evaluating the phytochemical analysis, a new approach to therapeutic and pharmacology.Alkaloids are hetrocylic nitrogen compounds, which are colorless and bitter in taste, also act as medicinal agents for their analgesic, antispasmodiac and bactericidal effects[3].

Flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage. Flavonoids are secondary metabolite produced in plants and act as defensive mechanism against microorganisms[4]. Saponins are glycosides of both triterpenes and steroids having bitter taste and foaming property[5]. Phenols are a member of group of aromatic chemical compound characterized by hydroxyl (OH) group attached to an aromatic ring. Phenols are toxic to the development of pathogens[6] and scavenge free radicals[7]. Tannins are polymeric or phenolic substances capable of tanning leather or precipitating gelatin from a solution[4].

Aloe species are shrubby, succulent, perennial, pea green color plants belonging to *Asphodeloideae family*. *Aloe vera (Linn),* also known as *Aloe barbadensis* of family *Liliaceae. Aloe Vera* plant has a long history of therapeutic ability and therefore has been promoted to combat large variety of clinical conditions. *Aloe vera* is a succulent stemless plant growing upto 60-100 cm tall. The leaves are thick and fleshy green with white flecks on the upper and lower stem surfaces. Usually flowers are produced as spikes that reach upto 90cm tall, with a yellow tubular corolla 2-3 cm long[8]. The chemical composition of leaf pulp includes anthraquinones, anthrones, chromones, essential and non- essential amino acids, carbohydrates, proteins, lipids, enzymes, inorganic compounds, vitamins, and miscellaneous organic compounds[9-11]. Leaves of *Aloe rupestris* are thick, shaggy, deep green, unspotted and bordered by red teeth and form a spiraled rosette at the stem apex in old plants. 7-O-methylaloesin is a natural product isolated from the leaf exudate of *Aloe rupestris*[12]. *Aloe juvenna* is a dwarf aloe with multibranching stems and forms smallish spikey rosettes from base. *G. pulchra* is very decorative, tall, narrow shrubby succulent with long finger-like marbled foliage. Separate studies data are available on the phytochemical study of *Aloe barbadensis*, but no reports of systematic studies on phytochemicals and comparison are there on *Aloe rupestris, Aloe juvenna* and *Gasteria pulchra*. The present work was aimed to evaluate the qualitative and quantitative analysis of phytochemicals in selected *Aloe species.*

2. MATERIALS AND METHODS

2.1 Collection and extraction of plant specimens.

Four different *Aloe* specimens such *as Aloe barbadensis, Aloe rupestris, Aloe juvenna and Aloe maculata* var. *pulchra (Gasteria pulchra)* were collected from different plant nurseries of Ghaziabad (Uttar Pradesh) and Alwar (Rajasthan) in India.Fresh leaves were dissected and washed properly with cold running tap water to remove the dust particles. Again it was rinsed in hot water to sterilize them and allowed to dry. The plant materials were air dried at sun shade for 1 week. 10g of each dry powdered plant materials were soaked in 100 ml of ethanol for 48 hrs. After 48 hrs of incubation, the plant extracts were filtered through Whatmann's filter. The extracts were evaporated in a waterbath at 55° C to yield crude extract and stored at 4°C for qualitative screening of phytochemicals.

2.2 Phytochemical screening

Preliminary screening of phytochemicals was performed by using standard procedures reported in literature [4, 13,14].

2.2.1 Test for Alkaloids

Few drops of freshly prepared Wagner's reagent were added to 1 ml of the sample, a brown precipitate indicates the presence of alkaloids.

2.2.2 Test for Cardiac Glycosides (Keller-Killiani Test)

To 0.5 g of plant extract was diluted in 5 ml of water, 2 ml of glacialacetic acid containing one drop of ferric chloride solution was added to the plant extract solution. This was underlayed with 1 ml of sulphuric acid. A brown ring at the interface may appear which indicates the presence of a deoxysugar characteristic feature ofcardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may formjust above the brown ring and gradually spreadthroughout this layer.

2.2.3 Test for Flavonoids

There were three methods to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract and concentrated sulphuric acid (1 ml) was added to it. A yellow colouration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. Then the mixture was filtered off. 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

2.2.4 Test for Phenolic Compounds

0.5g of plant extracts were dissolved in 5 ml distilled water. Few drops of neutral 5% ferric chloride solution was added to the plant extract solution. A dark green colour indicates the presence of phenolic compounds.

2.2.5 Test for Reducing Sugars (Fehling's Test)

The aqueousethanol extract (0.5 g of plant extract in 5 ml of distilled water) was added to boiling Fehling's solution (A and B) in a test tube. Formation of yellow or brownish red gives the positive result.

2.2.6 Test for Saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The froth solution was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formulation of emulsion.

2.2.7 Test for steroids

The crude plant extract was taken in a test tube and dissolved with 10 ml of chloroform. Equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer of the test tube turns into red colour and sulphuric acid layer shows yellow with green fluorescence which indicates the presence of steroids.

2.2.8 Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for the formation of brownish green or a blue-black colouration.

2.2.9 Test for Terpenoids (Salkowski Test)

To 0.5 g each of the extract was added to 2 ml of chloroform. 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface indicates the presenceof terpenoids.

2.3 Quantitative phytochemical analysis

2.3.1 Methanol extraction of plant specimens

10 g of each plant powder was added to 100 ml of methanol and incubated for 24 hrs. The solution was filtered and the filtrate was collected. The solvent was evaporated in a water bath to yield crude extract and stored at 4°C. Phytochemicals were evaluated quantitatively in the mathanolic extracts of plant specimens by standard procedures.

2.3.2 Determination of Alkaloids

The analysis was done according to method reported in standard reference book[4]. 5g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.3.3 Estimation of Flavanoids

This method is based on the formation of flavanoids-aluminium complex whose absorptivity is maximum at 415 nm. The reaction mixture consists of 1 ml of plant extract in methanol (10mg/ml) was mixed with 100µl of 20% aluminium trichloride in methanol and a drop of acetic acid , then diluted with methanol with 5 ml. Incubated at room temperature for 40 minutes and absorption was read at 415 nm. Blank samples were prepared from 100ml of plant extracts, a drop of acetic acid, and then diluted to 5 ml with methanol.A set of reference standard solutions of quercetin(0.5mg/ml) in methanol at (20, 40, 60, 80 and 100 µg/ml) were prepared. The absorbance for test and standard solutions were determined against the reagent blank at 415nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg /g of extract[15-18].

2.3.4 Estimation of Total Phenolic Compounds

100 mg of the plant extract of the sample was weighed and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube and added 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na_2CO_3 solution. Then the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml). Total phenol content was expressed in mg /gm[19].

2.3.5 Determination of Total Saponins

20 g of grounded sample were put into a conical flask and 1 ml of 20% aqueous ethanol was added. The samples were heated in a hot water bath for 4 hrs with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrated solution was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated[20].

2.3.6 Estimation of Tannins

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteuphenol reagent, 1 ml of 35 % Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared. Absorbance for test and standard solutions were measured against the blank at 725 nm. The tannin content was expressed in terms of mg/g of extract[21-26].

2.3.7 Determination of Terpenoids

100mg of plant powder were taken and soaked in ethanol for 24 hrs. The extract was filtered and filtrate was extracted with petroleum ether. The ether extract was treated as total terpenoids[27].

3. RESULTS AND DISCUSSION

The result of preliminary screening of phytochemicals in different *Aloe species*ethanolic extracts revealed the presence of wide range of active phytochemical constituents in all the tested *Aloe species.* Phytochemicals such as alkaloids, cardiac glycosides, tannins, terpenoids, steroids, saponins, reducing sugars were uniformly distributed in *Aloebarbadensis, Aloe rupestris, Aloejuvenna* and *Gasteria pulchra*. But steroids were absent in *Aloe juvenna* and cardiac glycosides were absent *Gasteria pulchra* (Table 1).

Aloe barbadensis was potentially efficient in having higher alkaloid content, flavanoids,phenolics, tannins and terpenoids than other species. But saponin content was found to be higher in *Aloe rupestris*. No remarkable variation was found among the species (Table 2). From the samples analysed,*Aloe barbadensis* was found to have higher alkaloid,phenolics, tannins and terpenoids. But saponin was higher in *Aloe rupestris*. Last but not the least phytochemical content was exhibited by *Aloe juvenna*. From the data analysed, it can be stated that *Aloe species* contain all the phytochemicals in appreciable amounts (Fig 1).The amount of phytochemical substances varies from species to species, depending on the age, climatic factors and ecological conditions [28].

Phytochemical constituents are the biochemical compounds used as the precursors for the development of drugs [14]. The presence and absence of these depends upon the physiological and biosynthetic reactions taking place inside the cell, the environment itself rectifies these modifications [29]. Results of studied *Aloe vera* resembles with the findings reported in literature[30-34].Reports indicates that phytochemicals attributes the medical effect in plants[35]. Observations are there on the fact that higher bioactive compounds inducehepatoprotective activity against CCl4 induced hepatotoxicity in the methanolic extracts of *Dendrobium ovatum* (L.) *Kraenzl* [36] . Methanol is the solvent that extracted highest amount of phytochemicals when compared with other solvent extracts[37-38]. Phytochemical compounds of *Aloe barbadensis* contribute antimicrobial activity[39].

Studies on alkaloids of *Aloe barbadensis* showed amazing effect on humans leading to the development of powerful pain killer medications and elimination of human cancer lines[40,41]. Saponin supported the plant to be useful in medical field by managing inflammation[42]. Alkaloids, saponins and tannins were reported to have antibiotic activity in treating pathogenic strains[43]. Phenolic compounds and flavonoids were toxic to pathogens [6] malignant tumors, in inactivating carcinogens and inhibiting the expression of mutagens[44]. In medical field, terpenoids is used in the aromatherapy[45]. Polyphenols were known to have antibacterial, antibacterial, antiviral, anti-inflammatory and antineoplastic activity[46]. Flavanoidsare biochemical compounds which contibute the antioxidant activity and radical scavenging activity [47]. These relevant data signifies the plant material with these phytochemicals is efficient for therapeutic and pharmacology purposes. *Aloe species* is blessed with all the phytochemicals and marked to be efficient in sustaining in medical field.

Table 1: Qualitative phytochemical screening of active compounds in *Aloe species.*

Phytochemical compounds	ALOE SPECIES			
	Aloe barbadensis	Aloe rupestris	Aloe juvenna	Gasteriapulchra
Alkaloids	+	+	+	+
Cardiac glycosides	+	+	+	-
Flavonoids	+	+	+	+
Phenolic compounds	+	+	+	+
Reducing sugars	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	-	+
Tannins	+	+	+	+
Terpenoids	+	+	+	+

Table 2: Quantitative analysis of phytochemical compounds (mg/g) in *Aloe species*.

SPECIES	ALKALOIDS	FLAVANOIDS	PHENOLICS	SAPONINS	TANNINS	TERPENOIDS
Aloe barbadensis	23.83 ± 0.28	18.66 ± 0.57	14.26 ± 0.25	4.9 ± 0.52	4.53 ± 0.3	13.5 ± 0.86
Aloe rupestris	22.83 ± 0.2	18.16 ± 0.76	14 ± 0.43	5 ± 0.75	4.23 ± 0.32	11.83 ± 0.78
Aloe juvenna	21.26 ± 0.68	16.1 ± 0.36	13.5 ± 0.43	3.83 ± 0.64	4.33 ± 0.26	11.16 ± 0.28
Gasteria pulchra	23.1 ± 0.81	18.56 ± 0.51	13.8 ± 0.45	4.23 ± 0.35	4.5 ± 0.43	12.46 ± 0.5

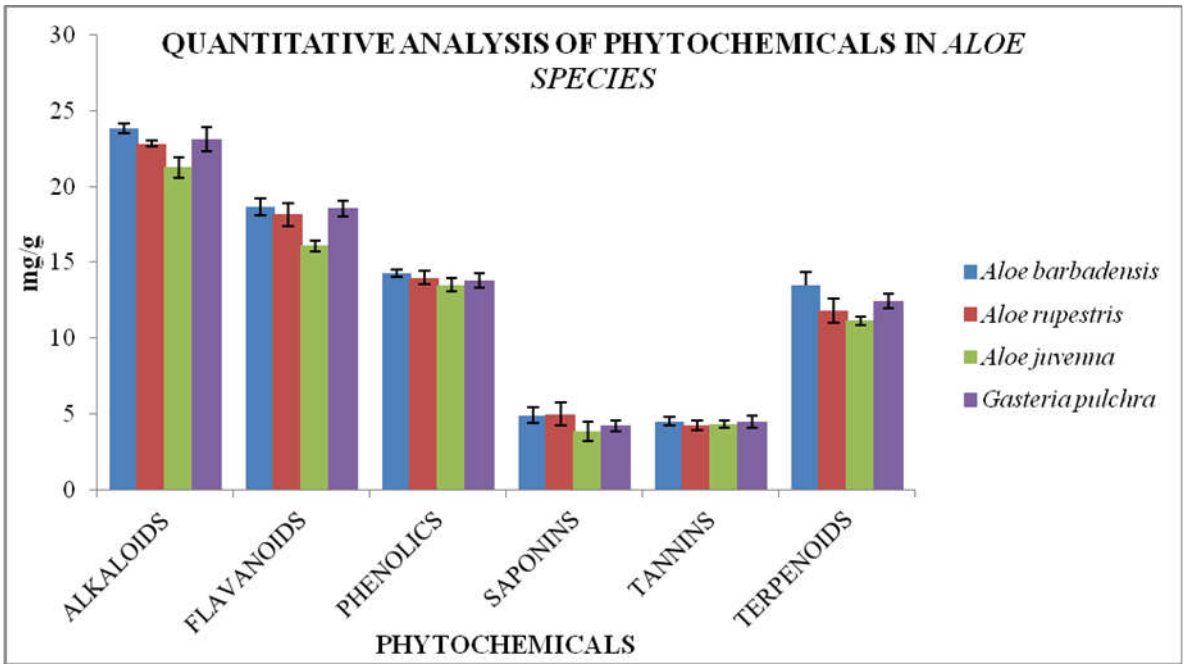

Figure 1: Quantitative analysis of phytochemicals in methanolic extracts of *Aloe species*.

4. CONCLUSION

Aloe species screened for qualitative and quantitative phytochemicals study seemed to have all biologically important secondary metabolites that can be used as potential source for the development of useful drugs against microbial infections and beneficial for good health. The results of present work also justifies that besides *Aloe barbadensis*, the other three species*, Aloe rupestris,Aloe juvenna* and *Gasteria pulchra* is equally talented in having secondary metabolites with slight variation.

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