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RESEARCH PAPER

A study on phytochemical extraction of *Aloe vera*

Beenu Singh Chauhan*, Albha Tiwari[1] **and** Arti Bhadauria
Department of Home Science, Jiwaji University, Gwalior (M.P.) India
(Email: bee05aug@gmail.com; artibhadauria1989@gmail.com)

Abstract : Aloe vera is considered to evaluate over its properties to alleviate the dislipidemic and hyperglycemic conditions on animal models to raise fresh evidences, so that theplant or its extracts could be suggested confidently for its use for health endorsing potential medicinal plant. The preliminary phytochemical analysis of hydroethanolic extract of *Aloe vera* showed presence of phenols, tannin, steroids, terpenoids and glycosides. Total Phenol Content (mg GAE/g), Total Flavonoid Content (mg QE/g) of hydroethanolic extract of *Aloe vera* were, respectively 393.65 mg and flavonoid 334.947 µg/mg, respectively.

Key Words : Phytochemicals, *Aleo vera*, Phenol, Flavonoids, Hydroalcoholic extract

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INTRODUCTION

Plants have a long history of use as traditional remedies to treat a variety of diseases and produce a range of diverse chemical scaffoldthat have specifically evolved to interact with biological targets. Many drugs based on plant-derived chemicals are currently employed in modern therapy (Seidel, 2020). And in this modern era medicinal plants are still remains of importance as a primary healthcare mode for approximately 85% of the world's population and as a resource for drug discovery, with 80% of all synthetic drugs deriving from them (Bauer and Brönstrup, 2014 and Fitzgerald *et al*., 2020).

Medicinal plants are the richest bio-resource of drugs for traditional systems of medicine. Since evolution, man has been using plant extracts to improve his health and life-style.

Ethnomedicinal uses, supported via way of means of medical evidences are critical for making sure secure and effective usage of herbal medicines.Bidkar *et al.* (2011) studied etnobotanically the use of *Cocus nucifera* shell ash has to determine the inhibitory effect of the water extract of *C. nucifera* shell ash on oral microflora from human being. Theirobservations from such a preliminary investigation describes the inhibitory potential of aqueous extract of *C. nucifera* Shell ash.

Aloe species (family Asphodelaceae) are among the most widely used plants over centuries for treating various ailments, for esthetic, and skincare (Chikezie and Ojiako (2015). The *Aloe* genus comprises over 430 species including *A. vera* and *A. ferox* among others (Salehi *et al.*, 2018). These species have been reported to have pharmacological activities including anti-inflammatory, immunomodulatory, antibacterial, antifungal, antiviral, antiproliferative, antidiabetic, laxative,

***Author for correspondence:**
[1]Department of Home Science, Govt Jhalkaribai College, Jiwaji University, Gwalior (M.P.) India

wound healing, moisturizing, anti-aging, and skin protection (Surjushe *et al.*, 2008).

Aloe species are increasingly being incorporated into different cosmetic products, health drinks, foods and beverages due to the above mentioned beneficial biological activities of the phytochemicals found mainly in the leaves (Sharma *et al.*, 2014).

These phytochemicals include polysaccharides, flavonoids, carbohydrates, coumarins, tannins, chromones, alkaloids, anthraquinones, organic compounds, pyrones, phytosterols, anthrones, sterols, vitamins, proteins and mineral constituents (Banik and Sharangi, 2019). The variation in concentration of these chemical constituents is based on the plant part used, extraction process, solvent, stage of growth and plant source.

Thus, the aim of this study was to evaluate the phytochemical constituents in hydroethanolic extract of *Aloe vera*.

MATERIAL AND METHODS

Collection of plant material :

Collection of plant material in present study was done from the local nurseries of Bhopal. The plant material chosen in present study were considered based on recent researches and available literatures regarding their medicinal and therapeutic potential and their use as curative herbal remedies with reference to folks, regional knowledge and Ayurveda.

The well identified plant materials were collected for the present study from Ahmadpur Nursery of Forest Department at Bhopal.

Extraction of phytochemical and screening :

For present study, the areal parts of the *Aloe vera* plant were used. The plant materials after collection, identification and approval were subjected to cleaning, shade drying and pulverization into fine powder before the process of extraction starts.

Defatting of plant material :

Before applying extraction, the powdered plant materials were defatted by soaking it in petroleum ether at room temperature for 24 hours to remove any fatty, oily or lipid content from them. After defatting of plant material the petroleum ether was remove by filtration and the crud drug is again dried that is to be extracted with distilled water and ethanol.

Soxhlet extraction:

Like the aqueous extraction about 10-20 grams of defatted dried powder was subjected to soxhlet extraction with 200 ml of 65% ethanol as extraction solvent till the complete exhaustion of sample material at 65°C. The defatted fine powders of *Aloe vera* leaves were subjected to soxhlet extraction with 65% ethanol till its completer exhaustion.

The extract so obtained after the process of soxhletion was subjected to evaporation of solvent to get the extract in form of crystals, slurry or paste. This is done by taking the extracted drug containing solvents in a glass beaker and placing them in a boiling water bath. The contents were kept in boiling water bath till the solvent of extract is evaporated completely.

The phyto extracts so obtained after this are now could be used to assess the yield of phytochemical extraction, evaluation of organoleptic properties, phytochemical analysis and other biological or pharmacological studies.

After concentration of phytochemical extracts both aqueous and ethanolic the organoleptic properties of extracted drug were evaluated. The extracted drugs were evaluated for colour, texture, smell and yield of extraction.

RESULTS AND DISCUSSION

The phytochemical extraction of leaves of *Aloe vera* was done successfully by drying them in room temperature and grounding them into fine powdered material to increase its surface area to allow maximum solvent content. These powder was then extracted with 65% ethanol as a solvent system to extract polar phytochemical in polarity solvents. The whole process was done successfully in 200 ml capacity Soxhlet apparatus for 5 days till the complete exhaustion of the crud plant material. In terms of chemical extraction using 65% ethanol as solvent of high polarity, the extract obtained using soxhletion process when concentrated when evaluated for percentage yield of extraction and organoleptic properties; the observations taken are depicted in Table 1 as results.

Table 1: Organoleptic properties of aqueous extract of aloe vera

Sr. No.	Variables/Quality	Properties
1.	% Yield	58.87%
2.	Colour	Brown
3.	Texture	Sticky
4.	Smell	Strange

The formulae used for percentage yield of extraction :

$$\% \text{ yield} = \frac{\text{Weight of extact}}{\text{Wight of crud subtract take before extraction}} \text{x100}$$

The organoleptic properties that include the properties of any extract like colour, texture or appearance and smell are taken by visual and sensory assessment, photographs are also available as records.

A small amount of the prepared extracts have been subjected to the preliminary phytochemical screening by adapting Harbourne's (1983) methods to check out the presence of the possible bioactive chemical groups including alkaloids, flavonoids, glycosides, tannins, terpenoids and saponins.

Table 2 : Phytochemical analysis of aloe vera extracts from leaves

Sr. No.	Constituents	Ethanolic extract of aloe vera leaves
1.	Alkaloids	+5
2.	Tannins	+2
3.	Terpenoids	+2
4.	Saponins	+2
5.	Flavonoids	+5
6.	Glycosides	+3

[(+) means present and (-) means absent]

In terms of qualitative screening of phytochemical groups present the ethanolic extract of *Aloe vera* leaves, it is reported that the extract prepared in present study are highly rich in various types of phytochemicals. These phytochemicals are generally responsible for various biological and pharmacological activities in providing health benefits for humans as nutritional and medicinal ingredients. Typically, such compounds are produced and accumulate at various levels in plant tissues (Nigg and Seigler, 2013).

According to Suresh and Abraham (2020) these plant secondary metabolites being beneficial for plants not only protecting them from UV irradiation, insects and extreme temperatures but also possess antioxidant, antibacterial, antifungal, anticancer, antimalarial, antiprotozoal, properties, etc. Phytochemicals with bioactive capability like salicylates observed withinside the willow tree bark for lowering inflammation, lycopene – a phytochemical observed in tomato to combat towards cardiovascular diseases and lung cancers. Hence, phytochemicals with novel structures may be exploited within the area of medication for brand spanking new drug discovery.

In present study when hydroethanolic extract of Aloe vera was evaluated in terms of present of various phytochemical constituents through phytochemical tests, it was reported to be positive for the constituents subjected to detection which are alkaloids, tannins, terpenoids, saponins, flavonoids and glycosides.

Test for alkaloids:

The diluted extract warmed for two minutes, filtered and few drops of dragendoffs reagent when added, orange red precipitate indicates the presence of alkaloids. Alkaloids are basic nitrogenous compounds with specific physiological and pharmacological activity. The extract sample that contains alkaloid produces white yellowish precipitate whilst some drops of Mayer's reagents are added (Siddiqui and Ali, 1997).

Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Evans, 2002).The alcoholic extract was evaporated to dryness and the residue was heated on a boiling waterbath with 2% hydrochloric acid. When reaction mixture comes down to the room temperature it is subjected to filtration and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation. The test of the presence of alkaloids was found positive in hydroethanolic extracts of *Aloe vera* leaves.

Test for tannins :

Small quantity of extracts mixed with water, heated, filtered and ferric chloride added. A darkgreen solution indicates the presence of tannins. Tannins were found to be positive in hydroethanolic extracts of *Aloe vera* leaves.

Test for terpenoids :

The diluted extract when mixed with 2ml chloroform ($CHCl_3$) and concentrated H_2SO_4 (3 ml) wash carefully added to form a layer. A reddish brown coloration of the interface formed indicating the presence of terpenoids. The test of the presence of terpenoids was also be positive in hydroethanolic extracts of *Aloe vera* leaves.

Test for saponins:

The small amount of extract stock was taken in test tube and diluted with 5ml of distilled water and then heated to boil which was followed by rigorous shaking lead to the formation of froth (appearance of creamy

mix of small bubbles) shows the presence of saponins. Sufficient amount of saponins are present in the hydroethanolic extracts of *Aloe vera* leaves reported through froth test.

Test for flavonoids:

Flavonoids are structurally diverse secondary metabolites in plants, with a multitude of functions. Because of their prevalence in the human diet, many flavonoids constitute important components of medicinal plants and are used in the control of inflammation and cancer prevention (Mathesius, 2018). In present work, the use of lead acetate test indicated the presence of rich amount of flavonoids hydroethanolic extracts of Aloe vera leaves.

Test for glycosides:

Glycosides are compounds which upon hydrolysis yields one or more glycones and aglycone or genine *i.e.,* the compounds which are sugars or non-sugars, respectively (Chhetri *et al.*, 2008). From the results of the benedicts test, the glycoside in hydroethanolic extracts of *Aloe vera* leaves.

Total flavonoids content (TFC) estimation :

The results of total flavonoid content estimation in hydroethanolic leaf extracts of *Aloe vera* in present study is described in this section. Total flavonoids content was calculated as quercetin equivalent (µg/mg) using the equation based on the calibration curve:

Y=0.040X + 0.009, R2 =0.999,

where, X = absorbance and Y = quercetin equivalent (QE).

Table 3: Quercetin as standard concentration vs absorbance at 420 nm to plot standard curve for estimation in samples using AlCl3 precipitation method

Sr.No.	Concentration (µg/ml)	Absorbance (λ)
1.	625	0.087
2.	1250	0.151
3.	2500	0.296
4.	5000	0.671
5.	10000	1.280

Instrument used: Single beam visible range digital micro-processed spectrophotometer from Electronic India model EI-2305

For estimation of total flavonoidal concentration, the 100 mg/ml stock solution of hydroethanolic extract of Aloe vera leaves was used to dilute with distilled water that results into 10 mg/ml concentration working samples extract for reaction. The absorbance after reaction for TFC analysis at 420 nm and its respective concentration are depicted in Table 4.

Table 4: Estimation of total flavonoid content in hydroethanolic extract of leaves of aloe vera in present study

Sr. No.	Sample extract	Absorbance of samples at 420 nm	Conc. in reaction sample	Actual conce. in µg/mg
1.	*Aloe vera* leaf hydroethanolic extract	0.429	3349.47 µg/10 mg	334.947 µg/mg

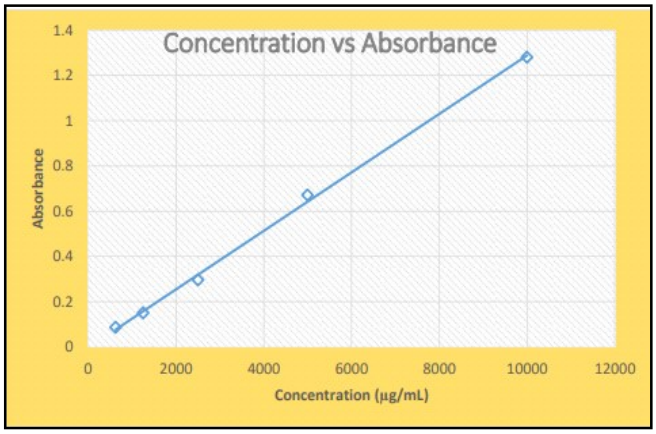

Fig. 1 : Standard plot for known concentration of quercetine standard. The graph is obtained from excel 2013 linear regression function

The total flavonoidal concentration equivalent to quarcetine structures is 334.947 µg/mg of extract which is supposed to be too rich to impart any biological and therapeutic effects which is the matter of further extensive investigation. But in present time very little of say almost no work is available in context to investigation on *Aloe vera* plant in order to generate any scientific data regarding the therapeutic significance of this plant species.

Total polyphenol content estimation:

The results of total phenolic content estimation in hydroethanolic leaf extracts of *Aloe vera* in present study is described. Total phenolic content was calculated as gallic acid equivalent (µg/mg) using the equation based on the calibration curve:

$Y = 0.0021X + 0.023$, $R^2 = 0.9909$

where, X = absorbance and Y = Gallic acid equivalent (GA).

Table 5: Gallic acid as standard concentration vs absorbance at 650 nm to plot standard curve for estimation of phenolics in samples Using Folin Coeucaltue's Method

Sr. No.	GA concentration in mg/ml	Absorbance at 650 nm
1.	2	1.891
2.	1	0.976
3.	0.5	0.457
4.	0.25	0.228
5.	0.125	0.128

Instrument used: Single beam visible range digital microprocessed spectrophotometer from Electronic India model EI-2305

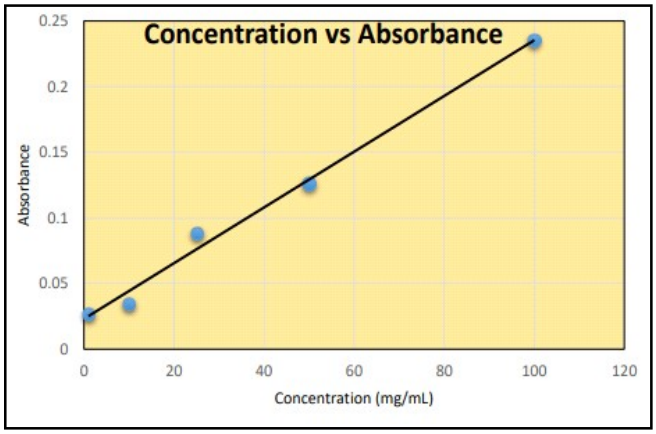

Fig. 2 : Standard plot for known concentration of gallic acid standard at 650 nm. The graph is obtained from excel 2013 linear regression function

For estimation of total phenolic concentration, the 100 mg/ml stock solution of hydroethanolic extract of Aloe vera leaves was used to dilute with distilled water that results into 10 mg/ml concentration working samples extract for reaction. The absorbance after reaction for TPC analysis at 650 nm and its respective concentration are depicted in Table 6.

Table 6: Estimation of total phenolic content in hydroethanolic extract of leaves of aloe vera in present study

Sr. No.	Sample extract	Absorbance of samples at 650 nm	Conc. in reaction sample
1.	Aloe vera leaf hydroethanolic extract	0.866	393.65 mg/ml

The total phenolic concentration equivalent to gallic acid structures is 393.65 mg/ml of extract in reaction mixture which is supposed to be too rich to impart any biological and therapeutic effects which is the matter of further extensive investigation. But in present time very little of say almost no work is available in context to investigation on *Aloe vera* plant in order to generate any scientific data regarding the therapeutic significance of this plant species.

Food and eating environs probably contribute to the rising epidemic of obesity and chronic diseases, over and above individual elements including knowledge, skills, and motivation. The most effective approaches among are environmental and policy interpolations for inculcating improvement in eating throughout the population.

Stressing an ecological structure for hypothesizing the various food environments and situations that have an effect on meals choices, with an emphasis on cutting edge information concerning the home, work sites, baby care, school, retail store and eating joints.

From the past century, obesity has appeared as prominent worldwide health issue via changes in current society and environment, favoring a positive energy balance and weight gain.

The concept of being on a "diet" for a chronic situation throughout life like diabetes is sufficient to position many humans off as understanding what to devour and retaining an ideal eating decorum are challenging. Medical dietary therapies became presented to manual a scientific and proof based tactic to the control of diabetes via diet, and its efficiency has been confirmed (Pastors *et al.*, 2002).

Although diabetes guidelines available mostly, suggests initiating pharmacotherapy only after making changes in lifestyle especially in nutritional intake and physical activity, which is generally not practiced worldwide. Most physicians being untrained in nutrition advising which in another hindrance in proper counseling of patients (Manson, 2017).

Myocardial infarctions and strokes are normally because of such blood clots. Furthermore, the atherosclerotic blood vessels are usually susceptible and might burst. So prevention is the best remedy in illnesses including atherosclerosis. Therefore, traditional clinical approaches usually focus on the changes in the lifestyle, including dropping the intake of saturated fats, cardio exercise and leaving the habit of smoking. Drugs also are used to decrease levels of cholesterol or blood pressure; however, most of the drugs have got substantial side effects (Bahmani *et al.,* 2015).

Approx. ten thousand phytochemicals have been identified, so far and lot more opportunity is still there for new discoveries. These recognized phytochemicals include flavones, tannins, alkaloids, triterpenoids, steroids,

and saponins.

Euterpe oleracea pulps also have got flavonoids that impart antioxidant potential by scavenging oxygen free radical.

The concentrations of polyphenols in food can be influenced by variety of plant, geographic region, season of cultivation, and storage. Dietary polyphenols could have 5 classes: phenolic acids, stilbenes, flavonoids, tannins and coumarins. Flavonoids can be further split into categories such as flavones, flavanones, flavonols, flavanols, anthocyanidins, and isoflavonoids.

Harinisri *et al.* (2019) while working with phytochemicals of Averrhoa carambola aimed at elucidate the antimicrobial and cholesterol-lowering effects of fruit of this plant. They analyzed the binary compound extract of fruit for varied phytochemicals in ripe fruit. They reported that the extracts of ripe fruits of *A. carambola* shows potential inhibitory activity towards B*acillus cereus, Escherichia coli, Klebsiella pneumonia, Pseudomonas* sp. and *Staphylococcus aureus* in the range of 13 to 20 mm zone size at 100 µl concentration when evaluated in vitro by disc diffusion method where the extracts contains the variety of phytochemicals including alkaloids, amino acids, proteins, carbohydrate, flavonoids, fixed oils, lipids, phenols, reducing agents, saponins, steroids and tannins. They reported ascorbic acid content 3. 538mg/ml while carbohydrate and protein contents as 300mg/ml and 110mg/ml, respectively. They also observed total flavonoid content and total phenolic content in fruit approx. 80m g/ml and 115mg/ml, respectively while the IC50 value in terms of DPPH antioxidant activity was reported to be 24.8µg/ml for alcoholic extract. Lastly, the most important they observer the cholesterol lowering potential of the extract using Zak's method using fatty food samples like artificial cholesterol, egg yolk and ghee in different concentrations (100-900mg/ml).

Conclusion:

Upon phytochemical extraction and analysis of *Aloe vera* plant material soxhlation method has proved to be worthy option for preparation of its hydro alcoholic extracts that resulted in a sufficient yield of extract which was rich in variety of phytochemical constituents including alkaloids, tannins, terpenoids, saponins, flavonoids and glycosides. Where at total of 334.947 µg/mg and 393.65 mg/ml of quercetine equivalent falvonoids and gallic acid equivalent phenolics were reported to be present in the obtained *Aloe vera* extract which is supposed to be an enough quantity of bioactive constituents to impart any biological, pharmacological or food supplemental activity.

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