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ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENTS OF *ALOE VERA* LEAVES EXTRACT

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Aloe vera L. is well known succulent plant speciesfor its marvellous medicinal properties and more than 75 active medicinal components have been identified till date. It possesses synergistic action in alleviating many diseased conditions such as stomach ailments, gastrointestinal problems, skin diseases, diabetes and oxidative stress-related diseases. Therefore, the present research aimed to evaluate the phytochemical constituents, Thin-layer chromatography (TLC), total phenolic&flavonoid contents, and antioxidant activity of aqueous and methanolic extracts of *A.vera*. The powdered plant material was used for the aqueous and methanolic extraction. Boththe extracts were screened for the presence of various phytochemical compounds. Most of the phyto constituents were present in both the extracts. Five and four different bioactive components having different Rf values were observed in methanolic and aqueous extracts respectively. Flavonoid and phenolic content does not vary significantly in the methanolic and aqueous extracts. Further, comparable dose-dependent antioxidant activity was observed in both the extracts. So, the present investigation suggests that aqueous and methanolic extract has significant antioxidant activity and may be utilized as a potential source of the therapeutic agent against many diseases.

Keywords: *Aloe vera*, Antioxidant Activity, Flavonoid, TLC, Phytochemical analysis

Introduction

Plants have been an important source of medicine since ancient time. The World Health Organization (WHO) also reports that up to 80 percent of people still rely primarily on conventional remedies for their medicines, such as herbs.Further, over the past century, the phyto-compounds in herbs play an important role in the pharmaceutical industries[1]and these are natural bioactive compounds seen in whole parts of the plants such as root, stem, bark, leaves, seed and fruits.

A.vera L. is a member of Asphodelaceae (Liliaceae) family. It has more than 500 species but only a few are medicinally important and has been known.It has been used for centuries because of its health, beauty, medicinal and skincare properties. The name *A. vera* derived from the Arabic word "*Alloeh*" meaning "shining bitter substance," while "*vera*" in Latin means "true."It has a significant quantity of bioactive compounds such as flavonoids, terpenoids, lectins[2,3] tannins, sterols, bases, anthraquinones, fatty acidsmono- and polysaccharides (pectins, glucomannan, hemicelluloses), enzymes, salicylic acid, sterols (campesterol, β-sitosterol),vitamins (A,C, E, β-carotene, B1, B2, B3, B6, folic

acid, B12, choline) and minerals(calcium, chromium, copper, iron, manganese, magnesium, potassium, sodium and zinc, phosphorus)[4,5]. Moreover, it contains various biological activities that improve the bioavailability of co-administered vitamins and absorption of poorly absorbable drugs, and also possess purgative, anti-microbial, immune-stimulatory, wound healing, anti-inflammatory, anti-tumour, anti-oxidant and anti-diabetic activities[6,7].

Oxidative stress plays an important role in the progression of chronic and degenerative diseases such asageing, cancer, asthma, autoimmune disorders, and neurodegenerative and cardiovascular diseases[8].Antioxidants inhibit the oxidation of many substances. They have become a requirement for food products that are susceptible to this form of chemical change to avoid food deterioration due to oxidation. Although commonly used synthetic antioxidants (e.g., BHA, BHT, TBHQ) are highly efficient, there is an increasing market demand for natural ingredients in processed foods. That is why researchers have been looking for new sources of natural compounds with antioxidant activity for a long time[9,10].

The human body has many pathways to combat oxidative stress by generating antioxidants, either *in situ* or supplied externally by foods and/or supplements.By preventing and repairing damage caused by ROS (reactive oxygen species), antioxidants function as free radical scavengers and can therefore strengthen the immune system and help reduce the risk of cancer and degenerative diseases[11]. Exposure to synthetic antioxidants over the long time has caused damage tothe liver and kidneys and may lead to mutagenesis.As a result, there is a good potential for natural antioxidant sources, such as plant derivatives.The secondary metabolites, specifically phenolic and flavonoids, can reduce the generation of ROS and alleviate oxidative stress-induced chronic diseases.

In this present study, we planned to determine phytochemical constituents, phenolic and flavonoid content inthe aqueous andmethanolic extracts of*A.vera*. TLC and antioxidant activities were also determined and compared.

Material and Methods

Plant material collection:

Fresh *A. vera* leaves were collected from the garden of the Department of Biochemistry, Bundelkhand University, Jhansi in February 2018. The leaves were thoroughly washed under running tap water for 5 min and followed by distilled water. Leaves were chopped and shade-dried for 2 weeks. Dry leaves were crushed into a fine powder and stored for further extraction.

Extract preparation:

Soxhlet apparatus was used for both aqueous and methanolic extract. 55 gm and 25 gm of dry powder of *A.vera* were taken in 250 ml of 80% methanol and distilled water respectively, run the soxhlet apparatus at 55-60°C, until it gets colourless. The extract was filtered using Whatman No. 1 filter paper. The filtrate was collected in an airtight bottle and stored at 4°C for further analysis.

Phytochemical Analysis:

For the detection of qualitative analysis of secondary metabolites viz alkaloids, phenols, carbohydrates, reducing sugar, flavonoids, saponinsetc present in aqueous and methanolic extracts of leaves of *A.vera*was performed by using the different standard protocol described elsewhere[12].

Antioxidant assay:

The total antioxidant activity of both the extracts were assessed by Prieto et al[13]. 0.1 mL of various concentrations of the extract were added with 1 mL of reagent and incubated at 95°C for 90 min. The absorbance was measured at 695 nm. Draw the calibration curve with the reference of ascorbic acid as a standard.

Thin layer chromatography(TLC):

Both the extracts were tested using TLC plates coated with silica gel-G of 0.2 mm thickness. Butanol-acetic acid-water (4:1:1 v/v) was used as a solvent[14]. A fully developed coated plate was air-dried followed by heating for 20-25 minutes. The plate was sprayed with 0.2% freshly prepared ninhydrin solution to detect the spots. These spots were expressed by their retention factor (Rf).

$$\text{Rf value} = \frac{\text{Distance Travelled by the Solute}}{\text{Distance Travelled by the Solvent}}$$

Determination of total flavonoid contents (TFC):

The total flavonoids content was determined by Chantiratikulet al[15]. The flavonoid content was calculated using the quercetin equivalent (mgQE/g) as a reference. 100 µl of different dilution were mixed with 500 µl of distilled water and 100 µl of 5% sodium nitrate, allowed to stand for 6 minutes. Then 150 µl of 10% aluminium chloride solution was added and further rest for 5 minutes followed by the addition of 200 µl of 1 M Sodium hydroxide solution. The absorbance was recorded at 510 nm.

Determination of total phenolic content (TPC):

The total phenolic content was determined by the Folin- Ciocalteu method[16]and calculated as gallic acid equivalents (mg GAE/g). 100 µl of different dilutions were mixed with 500 µl of water and then with 100 µl of Folin-Ciocalteu reagent and allowed to stand for 6 minutes. Then 1ml of 7% sodium carbonate and 500 µl of distilled water were added to the reaction mixture. The absorbance was recorded after 90 minutes at 760 nm.

Results and Discussion

The phytochemical analysis of the aqueous and methanolic extracts of *A.vera* leaves shows the presence of alkaloids, flavonoids, glycosides, cardiac glycosides, reducing sugar, tannins, saponin, protein, amino acid, terpenoids and steroids (Table- 1).

Wagner's and Hager's test for alkaloids were positive for both the extract whereas Mayer's test showed its absence. Molish's test for carbohydrates, Fehling's and Benedict test for reducing sugar showed positive results in both the extracts. Alkaline reagent and lead acetate test for flavonoids showed its absence in aqueous extract while methanolic extract showed its presence with lead acetate test.Borntrager's test and Legal's test used for glycosides confirmation. Only the aqueous extract showed the presence of glycosides with Borntrager'stest. Keller Killani test was applied for the estimation of cardiac glycosides which is present only in the aqueous extract.

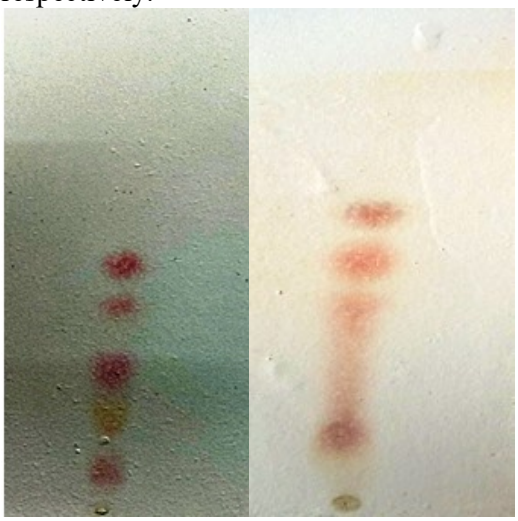
Table 1. Qualitative phytochemical analysis of the aqueous and methanolic extracts of *Aloe vera*

S. No	Photochemical Tests	*Aloe vera*	
		Methanolic extracts	Aqueous extracts
1.	Tests for alkaloids (A)Mayer's test (B)Wagner's test (C)Hager's test	 -ve +ve +ve	 -ve +ve +ve
2.	Test for carbohydrate (A)Molisch test	+ve	+ve
3.	Test for reducing sugar (A)Fehling's test (B)Benedict test	 +ve +ve	 +ve +ve
4.	Test for flavonoids (A)Alkaline reagent (B)Lead acetate	 -ve +ve	 -ve -ve
5.	Test for glycosides (A)Borntrager's test (B)Legal's test	 -ve -ve	 +ve -ve
6.	Test for cardiac test (A)Keller killani test	-ve	+ve
7.	Test for tannin and phenolic compound (A)Ferric chloride 5% (B)Lead acetate (C)Dilute iodine	 +ve -ve -ve	 +ve +ve +ve
8.	Test for saponin (A)Froth test	 -ve	 -ve
9.	Test for amino acid and protein (A)Ninhydrin test (B)Biuret test	 +ve -ve	 +ve -ve
10.	Test for triterpenoid	+ve	+ve
11.	Test for steroid	-ve	-ve

(+) indicates presence while (–) indicates the absence of the components

All tests for the determination of tannin and phenolics showed its presence in the aqueous extract. Lead acetate and dilute iodine tests showed the negative result in methanolic extract. Saponins possess detergent qualities that foam when mixed with water and was absent in both the extracts. Ninhydrin test has shown positive with both the extracts. Triterpenoids have been identified as anti-inflammatory, antiviral, antimicrobial, antitumoral, and as well as immunomodulatory agents. Triterpenoids are present but steroids are absent in both extracts.

Thin-layer chromatography (TLC) has shown a positive result for both extracts of *A.vera*. There were five and four spots having Rf values 0.05, 0.14, 0.24, 0.37, 0.48 (Fig. 1. (A)) and 0.23, 0.35, 0.47, 0.60 (Fig. 1. (B)) were observed in methanolic and aqueous extract respectively.



(A) (B)

Fig. 1.TLC plate showing spots having different Rf values (A) Each coloured spots showing different Rf value in methanolic extract of *Aloe vera*(B)Different Rf values of aqueous extract of *Aloe vera*.

The total antioxidant capacity of *A.vera* was determined and it has shown the dose-dependent activities (Fig. 2.). There are slight differences in terms of total flavonoid contents (TFC) in both the extracts and the mean value ± SE for methanolic and aqueous extract are 476±84.67 and 451±59.33, mgQE/g, respectively. The total phenolic content (TPC) in both the extracts were also not significantly different from each other and the mean value ± SE of the methanolic and aqueous extracts are 120±29.24 and 136±23.95 mgGAE/g, respectively (Fig. 3.).

Free radicals and reactive oxygen species (ROS) leads to various chronic diseases and the substances which are responsible for the protection are called antioxidants[17].Antioxidants are categorized into two groups such as enzymatic and non-enzymatic. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase, endogenously produced in human systems and under normal conditions they provide defence against the free radicals and ROS. However, antioxidant enzymes are weakened against radicals during severe disease conditions[18]. Therefore, the external sources of dietary antioxidants are in need to strengthen the human defence system. Polyphenols, carotenoids, vitamins, and minerals, which are abundant in fruits and vegetables, are non-enzymatic antioxidants[19]. Polyphenols comprise one of the most abundant and pervasive classes of plant metabolites that provide a broad spectrum of biological activities including antioxidant activity[20,21]. The antioxidant properties are typically determined by phenolic constituents[22]. Plant phenolic content is primarily responsible for their free radical scavenging mechanism[23].

The current research was hypothesized to investigate the methanolic and aqueous extracts of *A.vera* leaves as they possess a vast variety of phytoconstituents, as well as to ascertain their polyphenolic contents (TPC and TFC) and antioxidant activities. It has been reported that phenolic compounds are known to exhibit strong antioxidant activities, which have direct antioxidant properties due to the presence of hydroxyl groups, which act as hydrogen donor[24]. Additionally, they are found to be effective

in scavenging free radicals as a result of their redox properties that allow them to act as reducingagents[25]. Moreover, flavonoids are hydroxylatedphenolics and are potent water-soluble antioxidants that help in radical scavenging and the prevention of oxidative cell damage. They have been reported to possess strong antioxidant activities[26,27].A high concentration of flavonoid and phenolic content was observed in the whole leaf extracts of *A. vera*. The total phenolic and

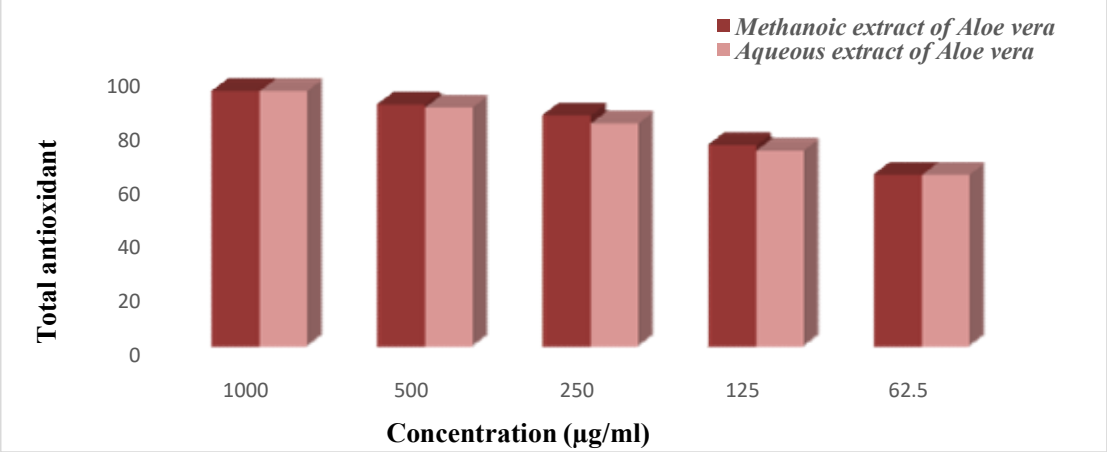


Fig. 2.The scavenging activity on phosphomolybdenum of methanolic and aqueous extract of *Aloe vera*

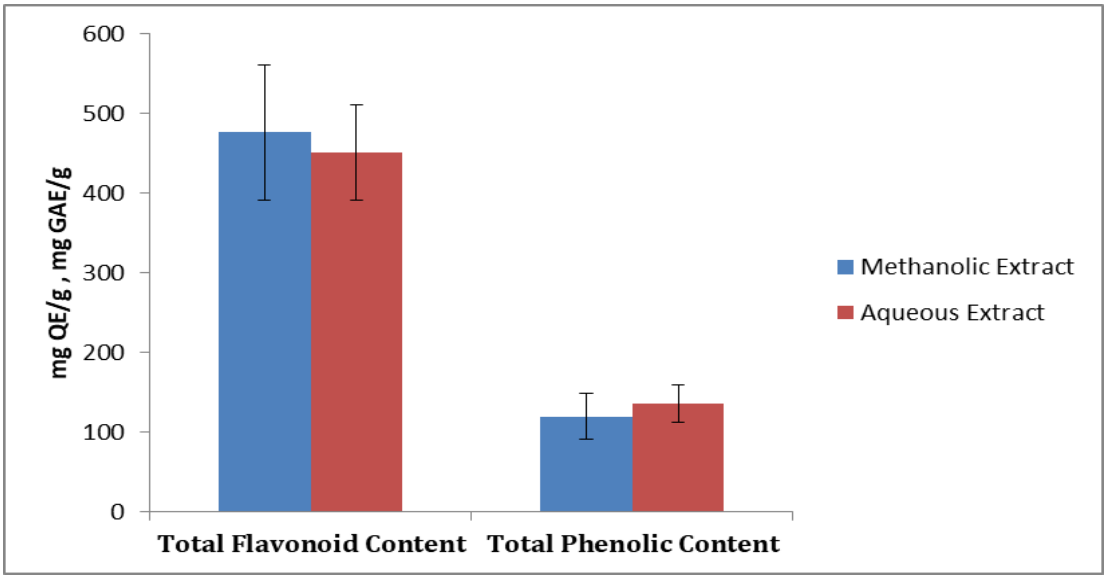


Fig. 3. Total Flavonoid and Phenoliccontents of methanolic& aqueous extract of *Aloe vera*

flavonoid content was comparable in both the solvent extracts investigated in this study.

Most antioxidant activities depend on the amount of phytochemicals present in the plants. Our results show the antioxidant activity in the leaves of *A.vera* extracts. Therefore, it may be possible to stop the oxidative development of a variety of diseases, including cancer, neurodegenerative and cardiovascular diseases, osteoporosis, and diabetes mellitus, using this information[28,29]. Since they are efficient in neutralizing ROS and alleviating oxidative stress, plant-derived antioxidants are considered to be a significant solution in delaying the prognosis of diabetes[30,31]. As a result,

plants with high enzyme inhibitory and antioxidant potential are considered likely a therapeutic candidate for diabetes management[32].Sazhina et al. (2016) reported that leaf extracts from 15 *Aloe* species exhibited high antioxidant activity[33]. Thus the plant had potent antioxidant properties to curtail the progression of radical related diseases and thereby give credence to the traditional usage of *A. vera* extract. The observed results suggest further analyses to confirm its prophylactic effect in the treatment of free radical-mediated diseases.

Conclusion

The present study showed high phenolic and flavonoid contents by aqueous and methanol whole leaf extracts of A. vera. Moreover, the aqueous & methanolic extract showed dose dependant antioxidant activities. Five and four bioactive components were observed in methanolic and aqueous extracts respectively. Further, research is required to evaluate the activity of the individual component. Although, based on the present finding we can conclude that the whole leaf extract could be used as a potent antioxidant in the medicine and food industries.

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