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Research Article

Determination of Phytochemical and Antioxidant Activities of *Aloe vera* Gel Extracts

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Abstract

This study aimed to determine the phytochemical and antioxidant activities of *Aloe vera* gel extracts. The phytochemical obtained by different methods(methanol macerated, methanol soxhlet and aqueous extract). The extract of *Aloe vera* Gel was subjected to primary phytochemical screening. Methanol macerated method revealed the presence of various phytochemicals. It resulted in the abundance of flavonoids, terpenoids, steroids, tannins, alkaloids, phenolic compounds, quinines, anthraquinones, and saponins. The results for the flavonoids, tannins and Alkaloids concentrations were 3.45, 1.20 and 2.60%, respectively. To investigate the antioxidant activity, *Aloe vera* Gel was successively extracted using *n*-hexane, dichloromethane and methanol. These extracts were analyzed using the DPPH radical scavenging method. The results found that the methanol extract is more effective in the free radical antioxidant (66.85%) followed by Dichloromethane (36.80%) and then Hexane (26.85%).

Keywords: *Aloe vera* Gel; Anti-Oxidant; Phytochemical; DPPH

Introduction

Medicinal plants are considered as the greatest pharmaceutical stores existing on the earth as they can produce secondary phytochemicals having bioactive properties. These phytochemicals work efficiently to cure various diseases and illnesses since ancient times [1]. The use of plant in the treatment of the diseases is as old as human civilization on the earth. Although it is not clear how the primitive people discovered the phenomenon that plants could be used to cure disease. Natural product can be defined as a chemical organic substance which is produced by the living organisms found in the nature that are produced by the path way of primary and secondary metabolism [2]. Even today the World Health Organization (WHO) estimates that up to 80% of people still rely on traditional remedies such as herbs for their medicine [3]. Aloe (*Aloe vera*) is an important and traditional medicinal plant belonging to the family Liliaceae. It is indigenous to Africa and Mediterranean countries [4]. *Aloe vera* has broad range of pharmacological properties, including anti-inflammatory, antiviral, antioxidant actions, antibacterial, antifungal, analgesic, antitumor, anti-diabetic and inhibition of tumor cells activation and proliferation [5]. The gel consists primarily of water (> 98%) and polysaccharides such as pectin, cellulose, hemicellulose, glucomannan, acemannan and mannose derivates [6]. *Aloe vera* gel also contains potentially active constituents, such as vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acid, and amino acids [6]. Many studies have demonstrated that *Aloe vera* has great properties and can be used as anti-inflammatory, antibacterial, anti fungal, anticancer and even antioxidant agent in these plants in Sudan. A great use of aloe gel was studied to be used as antioxidant. The search for newer natural antioxidants, wound healing, especially of plant origin has ever

since increase. So, this study was conducted to investigate the presence of phytochemicals in extracts of Aloe Vera gel and to evaluate the antioxidant activity for the extracts.

Materials and Methods

Plant material

The leaves of *Aloe vera* were brought from Garden Plant in Khartoum, Sudan. The leaves were cleaned, de-shelled and dried at room temperature. The gel was ground to powder using a grinder prior to extraction.

Chemicals and reagents

All chemical and reagents were in analytical grades.

Methods

Preparation of *Aloe vera* gel powder

Aloe vera gel powder was prepared according to the method described by Saritha., *et al*. [7] with some modifications. *Aloe vera* fresh leaves were scraped with sterilized spoon to remove the gel. Then the gel was blended in an electric blender. The blended gel was freeze dried and stored prior to further use.

Extraction methods

Preparation of methanol extracts

One hundred gm of dried pods powder of *Aloe vera* were transferred into beaker and solution of methanol (500 ml) was added, then the contents of the beaker were left at room temperature for three days with constant shaking; the extract was filtered through filter paper, left to dry for one hour at room temperature. The residual weight was recorded and yield(%) was calculated.

Preparation of water extract

One hundred gm of *Aloe vera* powder were transferred into a beaker and then 500 mg of water and two drops of acetic acid were added. The contents of the beaker were left at room temperature for three days. The extract was filtered through filter paper and left to dry at room temperature. The residual weight was recorded and yield (%) was calculated.

Preparation of macerated extracts

One hundred gm of *Aloe vera* was extracted by macerate method by successively solvents hexane, dichloromethane and methanol at room temperature. The extraction process was repeated till the solvents became colorless. The extracts were then filtered using Whatman No.1 paper. The filtrates were concentrated in vacuum at 50°C ± 1 in a rotary evaporator to obtain the crude extracts [7].

Phytochemical screening of *Aloe vera* extracts

The methanol and water extracts of pod were used for the following tests according to methods described by [8].

Quantitative determination of chemical constituency

The chemical constituency quantities (alkaloids, tannins and flavonoids) were achieved according to the method mentioned by Edeoga [9].

DPPH free radical scavenging activity

The antioxidant activity of *Aloe vera* gel freeze dried extracts (methanol extract, Dichloromethane extract and Hexane extract) was determined. Gallic acid and the standard compound BHA was measured in terms of hydrogen donating radical scavenging ability using the DPPH method. 0.1 ml of extract was added to 2.9 mL of methanol solution. After centrifugation, the supernatant is collected 50 µmol L^{-1} of DPPH solution is added. Kept in the dark for 45 minutes and the resulting decrease in absorbance at 517 nm were recorded against blank using a UV-Vis Spectrophotometer. Amongst test samples, MEAG and AEAG showed the maximum scavenging activity and hence were used for all the subsequent studies. The radical scavenging activity on DPPH was expressed as, Scavenging effect (%) = [(Ao - A1)/Ao] x 100 Where Ao is the absorbance of control and A1 is the absorbance of sample extract or standard [7].

Column chromatography

Methanol extract (2g) was subjected to column chromatography on silica gel (60 - 120 mesh - Merck) packed and eluted with mixture of Hexane, ethyl acetate and methanol of increasing polarity to obtain fractions. The admixture was packed on a silica gel column (Merck, India) and eluted started with 100% hexane and increased with solvent polarity ethyl acetate, and methanol, in the ratio of 90:10, 80:20, 70:30, 50:50, 30:70, 20:80, 10:90. (70:30) gave a colorless compound and on further purification with ethyl acetated and methanol the isolated compound yield.

Thin layer chromatography

Thin Layer Chromatography was carried out according to the method described by [10]. The activated percolated silica gel aluminum sheets TLC plates were placed on the laboratory bench and the active methanol extract was carefully spotted at about 2 cm

from the bottom of the plate. After air evaporation of the plate, the plate it was placed vertically on glass tank which contained suitable solvent to a depth of about 1.5 cm and the chromatogram developed. The plate was removed from the jar, allowed to dry, visualized by UV lamp, and finally sprayed with 1% vanillin. Retardation factor (RF) value of separated fractions was calculated as follow:

$$RF = \frac{\text{Distance traveled by spot}}{\text{Distance traveled by solvent front}}$$

Preparative thin-layer chromatography

A concentrated alcoholic extract solution was chromatographic on preparative TLC plate. The plates were twice developed in a mixture of (5:4:1) (toluene: ethyl acetate: formic acid), reagents different zones were located. Each was scrapped off in a separate container, and each zone was removed by washing the dry Silica gel obtained with (chloroform: methanol) (80:20) sel times, decanting the solvents and removing the excess Silica gel by filtration. The extracts were concentrated and the resulting solutions of different zones were passed separately through a sintered glass (porosity no3) to remove any trace of Silica gel. The 2 filtered extracts were then concentrated [10].

Results and Discussion

Phytochemical screening of *Aloe vera gel*

The results for Phytochemical screening of *Aloe vera* gel extracts using different solvent in different polarity and were represented in table 1. It showed that main secondary metabolites presence in this plant. Aqueous and methanol maceration extraction methods revealed the presence of the flavonoid, glucoses, saponin and carbohydrates, however, methanol soxhlet extraction method was not revealed these compounds, and this may be due to the effect of heat on some phytochemicals compounds. Alkaloid and phenol were found in methanol maceration extract, but, was not revealed in Aqueous and methanol soxhlet extracts. In addition, tannins and Anthraquinone were present in all extracts. Moreover, Quinine is present in the methanol maceration, but it's not present in methanol soxhlet and Aqueous extracts. Results for the presence of tannins, saponin, Alkaloids, flavonoids and Glycosides in methanol maceration were agreed with those found by Priyanka and Srivastav [11]. While the results for sterols were disagreed with Priyanka and Srivastav [11] who reported the presence of sterols in methanol maceration extract. Results for the presence of saponin, Alkaloids and flavonoids in methanol maceration were agreed with those found by Darshan., *et al.* [12]. While the results for sterols were disagreed with Darshan., *et al.* [12] who reported the presence of sterols in methanol maceration extract in the screening process of sterol gave positive results which is disagreed with my results of sterol, were gave- negative results of all extracted.

Test	Maceration Extraction Methanol	Soxhlet extraction Methanol	Aqueous
Alkaloid	+++	+	+
Tannins	+++	+++	+++
Phenol	+++	-	-
Sterol	-	-	-
Flavonoid	+++	++	+++
Glucoses	+++	-	-
Anthraquinone	+++	+++	+++
Protein	+	++	++
Quinine	++	-	+
Saponins	+++	++	+++
Carbohydrates	+++	++	+++

Table 1: General phytochemical screening of *Aloe.*

Key: + + +: Strong, + +: Medium, +: Weak, -: Negative.

Quantitative determination of Alkaloid, Flavonoid and Tannin chemical constituency of *Aloe vera*

Based on the results of phytochemical screening aloe vera gel extract, the quantities analysis of Alkaloid, Flavonoid and Tannin were shown in table 2. The results indicated that, flavonoids are the highest concentration followed by Alkaloids and tannins. These findings were similar to those reported by Adesuyi., *et al.* [3]; [flavonoids (3.25%) and Alkaloid (2.47%)]. On other hand the results for tannins are quite different from those published by Adesuyi., *et al*. [3]; [tannins (0.155%)].

Component	Weight (gm)	Yield (%)
Alkaloid	0.13	2.60
Flavonoid	0.35	3.45
Tannins	0.12	1.20

Table 2: Quantitative determination of alkaloid, flavonoid and tannin chemical constituency of *Aloe vera.*

DPPH radical-scavenging activity of *A.* gel extracts

The results of solvents extraction and antioxidant activity for Aloe vera gel extracts are shown in table 3. It was found that metha-

nol has achieved the highest extraction yield. Moreover, the methanol extract demonstrated the highest antioxidant activity compared to hexane and dichloromethane. Saritha V., *et al*. [7] found that the methanol extract exhibited (94%) for antioxidant activity which is higher than the finding of this study. Because different regions.

Saritha., *et al*. [7] the effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. Priyanka Das 1, Alok Kumar Srivastav (2013). The *Aloe vera* extracts are phenolic compound, glycosides, alkaloid and flavonoid. Among these phenolic compounds which may be responsible for the activities of antioxidant, Because of their strong capacity to donate electrons or hydrogen atoms.

Solvent	Extraction yield %	RSA-DPPH %
Hexane	3.58	26 ± 08
Dichloromethane	6.83	36 ± 58
Methanol	19.44	66 ± 48

Table 3: Anti-oxidant activity of *Aloe vera* gel.

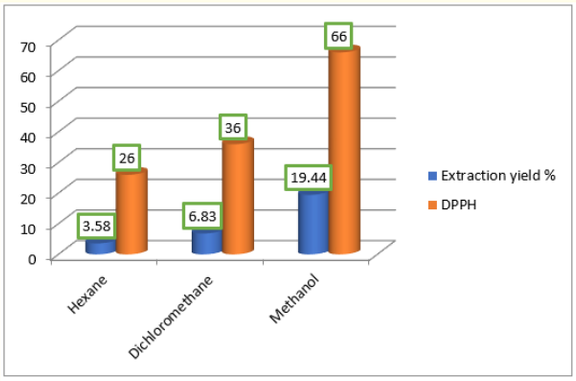

Figure 1: Antioxidant activity of extracts of *Aloe vera* gel.

Thin layer chromatography

The methanol extraction of *Aloe vera* gel, which showed the highest antioxidant activity was examined using thing layer chromatography (Table 4). The results of TLC for methanol extract showed that, the methanol extract consist of (4) fractions with different R_f value and different colors, then of fractions were detected under (UVλ366) nm, which some fractions gave the same color under (UVλ254) nm it may be isomer and spear.

Fraction	RF	Mobile phase	Colour			
			vis	UV (356)	UV (245)	Spear reagent
F1	0.27	5:4:1	Yellow	Yellow	Yellow	-
F2	0.44	5:4:1	-	-	-	Green
F3	0.61	5:4:1	-	-	Red	-
F4	0.77	5:4:1	Yellow		Yellow	

Table 4: Thin layer chromatography.

Column chromatography

The methanol extraction of *Aloe vera* gel, that showed the highest antioxidant activity was subjected to column chromatography (Table 5). Similar TLC spotting profiles were combined. The results of column chromatographic for methanol extract showed that, the methanol extract consist of (4) fractions, in each fraction consist of more fractionation, which are explained at the following: fraction 1 (1 - 32) fractionation, fraction 2 (33 - 50) fractionation, fraction 3 (51 - 70) fractionation and fraction 4 (71 - 100) fractionation, with different R_f value and different colors, then the No of fractions were detected under (UVλ366) nm and some of fractions gave the same colors under (UVλ 254) nm which it may be isomer and spear.

Fraction	Rf	Mobile phase	Colour			
			UV λ (254mm)	(360mm) λ Uv	Vis	Spray
F1 (1 - 32)	0.66	9:1	Yellow	-	Yellow	
	0.83		Blue	-	Yellow	
F2 (33 - 50)	0.34	8:2	Yellow	-	Yellow	
	0.50		Pink	-	-	
	0.71		Pink	-	Yellow	
	0.84		Blue	-	Yellow	
	0.86		Red	-	-	
F3 (51 - 70)	0.60	8:2	Yellow	-	Yellow	
	0.62		Blue	-	Yellow	
F4 (71 - 100)	0.45	7:3	-	-	-	Green
	0.61			-	-	Red pale
	0.72		-	-	Yellow	Blue
	0.81		-	-	Yellow	Yellow

Table 5: Characteristic of different fraction of column chromatography.

Phytochemical screening of factions for column chromatographic (test flavonoids)

Phytochemical screening of factions for column chromatographic are described in table 6 using different solvent in different polarity and showed main secondary metabolites presence in this plant. The result revealed the presence of flavonoid of fraction column. In the analysis of flavonoids compounds brownish Red colour was developed to indicate the presence of flavonoid, similarly based on the presence or absence of colour change which indicate positive or negative results. The fraction three (51 - 70) showed positive result, fractions 1 (1 - 32), fractions 2 (33 - 50) and fractions 4 (51 - 100) are showed negative results [13,14].

Fraction	Test / KOH	Colour	Test/H_2SO_4	Colour
F1	-	Yellow	-	Yellow
F2	++	Yellow Red	++	Yellow Red
F3	+++	Red	+++	Red
F4	-	Yellow	-	Yellow

Table 6: Phytochemical screening of factions for column chromatographic (test flavonoids).

Key: + + +: Strong, + +: Medium, +: Weak, -: Negative.

Conclusion

- *Aloe vera* gel is rich sources of phytochemical alcoholic and aqueous extracts containing diverse group of phytochemical such as flavonoids, terpenoids, steroids, tannins, Alkaloids, phenolic compounds, carbohydrates, coumarins, proteins, quinines, anthraquinones and saponins.
- The results obtained in this study clearly demonstrated broad spectrum anti-oxidant activity of *Aloe vera* gel extract. More important the result indicated that methanol *Aloe vera* gel extract more effective as antioxidant than the other extracts. The presence of phenolic compounds including phenols, flavonoid and tannins as major active constituents may be responsible for these activities.

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