

Phytochemical, Antioxidant and Antibacterial Studies of Ethanolic and Methanolic Extracts of *Aloe elegans* Leaves gel

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Abstract: The study was aimed at investigating the phytochemicals, antioxidant and antibacterial activities of the ethanolic and methanolic extracts of *Aloe elegans* leaves gel. The phytochemical screening revealed the presence of phenols, flavonoids, anthraquinones, tannins, saponins and terpenoids in both extracts. The antioxidant activities of both extracts were assayed using ferric reducing antioxidant power (FRAP), conjugated diene (CD) and peroxide value (PV) methods using sunflower oil (SFO) as the oxidizable substrate. The value for methanol and ethanol extracts of *Aloe elegans* leaves gel and the standard ascorbic acid in the ferric reducing power assay at 20 µg/mL were found to be 65.32%, 62.89%, 59.4% respectively. The peroxide value after 12 days at room temperature for the Sunflower oil (SFO) was found to be 45 mmol/L while the value for the substrate containing methanol, ethanol extract and vitamin E were found to be 36, 38, 43 mmol/L respectively. The value of the absorption coefficients of conjugated dienes resulted from oxidations of the methanol and ethanol extracts and vitamin E were found to be 3.006, 3.387, 4.306 g⁻¹cm⁻¹mL respectively. In all the antioxidant assays used, the methanolic extract was found to inhibit oxidation of sunflower oil to a better extent than its ethanolic counterpart. Relative to vitamin E, both extracts exhibited better antioxidant efficacy. In the antibacterial assay, both the extracts showed potent bactericidal effects against *Escherichia coli* and *Staphylococcus aureus*, though to a lesser extent than the standard antibiotic Gentamicin.

Key words: *Aloe Elegans*, Antibacterial Activities, Antioxidant, Phytochemicals.

1. Introduction

Aloe elegans (Synonyms: *Aloe abyssinica*, *Aloe vera* var, *aethiopica*, *Aloe abyssinica* var, *Aloe aethiopica*) is a member of *Aceae* family. It is a very short-stemmed plant which grows to 60-100 cm (24-39 inch) tall and has very thick and fleshy leaves with a color of green to grey-green. The perimetric bundle sheath cells give rise to bitter, yellow exudates [1]. *Aloe elegans* leaf has three layers, shown in Figure 1; the outer layer (Rind) which is composed of carbohydrates and proteins and acts as a protective layer. The middle layer has a yellow sap and is contained by anthraquinones and glycosides. The inner layer is a clear gel which contains 99% water

and the rest is made of amino acids, lipids, sterols, vitamins, enzymes, polysaccharides, phenolic compounds and organic acids [2-4].

The *Aloe elegans* plant is a rich source of many chemical compounds of biological significance. It is reportedly composed of ingredients like; vitamins, minerals, enzymes, sugars, anthraquinones, phenolic compounds, lignin, saponins, sterols, and amino acids. The numerous medicinal, nutritional, and cosmetic benefits of this plant can be traced to its chemical composition [5-8].

Living things always need a balance between the production and neutralization of reactive oxygen species (ROS). Unless the cells start to suffer the consequences of oxidative stress [9-12]. On an average estimation, a human cell is attacked by the hydroxyl and other radical species at an average rate of 10⁵ times

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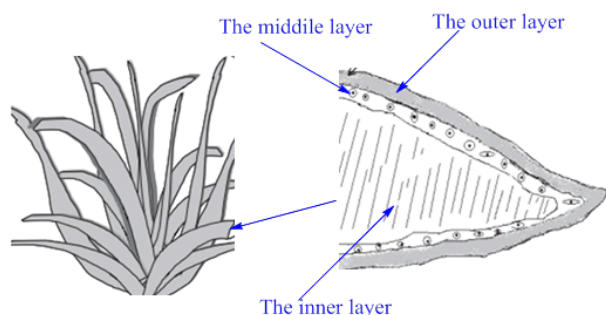


Fig. 1 The three layers of *Aloe elegans* leaf plant.

inducing oxidative stress [13]. Mainly the DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) molecules, sugars and lipids parts of a cell are the ones targeted [12]. Effect such as; production of base-free sites, deletions, modification of all bases, frame shifts, strand breaks, DNA-protein cross-links and chromosomal arrangements are visibly observed. As a result, the reaction during DNA damage produces hydroxyl radical [14]. Sugar is also believed to form oxygen free radicals which do result in glycoxidative damage [15]. Lipids are forced to form peroxy radicals [16].

Knowing these all, antioxidants which do prevent the free radical formations are therefore extremely necessary. An antioxidant is any substance which decreases or resists the oxidation of another substance by sacrificing itself acting as an oxidizable substrate. The free radicals formed are stabilized by factors like resonance [17-20]. This antioxidant activity happens in ways such as; inhibition of free radicals, interruption of propagation of the autoxidation chain reaction, through synergism with other antioxidants, converting hydroperoxides into stable compounds, acting as a metal chelators by converting metal pro-oxidants into stable products, and inhibition of pro-oxidative enzymes. Human being has both enzymatic antioxidants and non-enzymatic oxidants systems [21-28].

Food and fragrance industries use synthetic antioxidants to protect the products from oxidative deterioration as well as increase their shelf life, even though there are some concerns over their negative

health effects. These antioxidants include BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) [29-30].

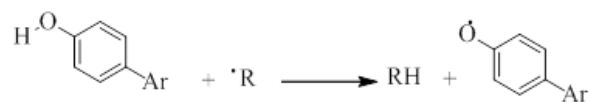
Antioxidants do deactivate free radicals through mechanisms including hydrogen atom transfer (HAT) and single electron transfer (SET). These two mechanisms are depicted for phenolic antioxidants in Schemes 1 and 2, respectively [31].

It is assumed that the newly formed free radical $\text{ArO}^\bullet$ and $\text{ArOH}^{\bullet+}$ are expected to be more stable than the free radical (R) quenched by the phenolic antioxidant. Flavonoids are one of the efficient free radical scavenger phytochemicals [30-34].

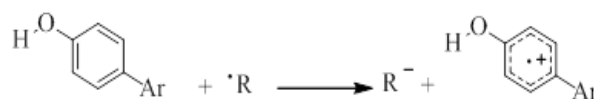
There are various antioxidant activity assays methods used having their own advantages and disadvantages. No method can provide unequivocal results. Hence the best option is using a variety of methods. Some of them use synthetic antioxidants or free radicals while others use animal or plant cells.

One of the most commonly used parameters to evaluate oil and fatty food oxidation, by iodometric titration, is peroxide value determination [35, 36]. The peroxide value is determined by measuring the iodine liberated from potassium iodide by peroxide, using sodium thiosulfate solution as the titrant. The reactions which taking place in the presence of acetic acid are shown in Scheme 3.

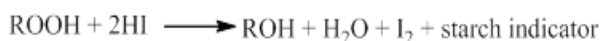
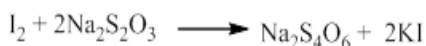
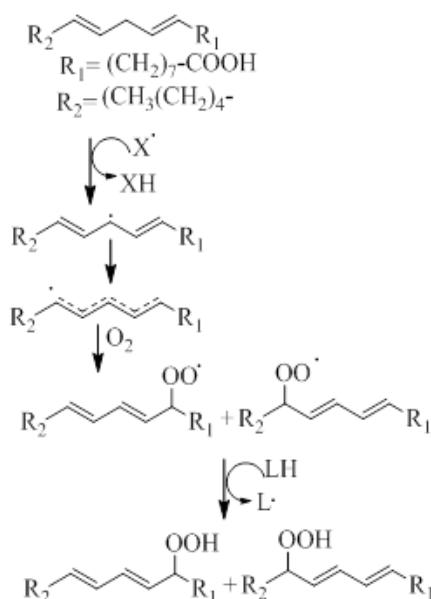
Diene conjugation is also another useful technique for the study of lipid oxidation. Diene conjugation is an outcome of lipid oxidation and is now utilized as an end-point for the determination of the antioxidant activity of a sample. The usual is a substance containing



Scheme 1 Antioxidant mechanism involving hydrogen atom transfer.



Scheme 2 Mechanism involving single electron transfer.

Generation of hydroperoxideGeneration of iodineTitration step**Scheme 3** Reactions occurring during peroxide value determination.**Scheme 4** Conjugated diene formation following lipid peroxidations.

polyunsaturated fatty acids, with oxidation being initiated. Determining the quantity of the conjugated dienes is done by calculating the increase in absorbance per mass of sample at a fixed time [37-44].

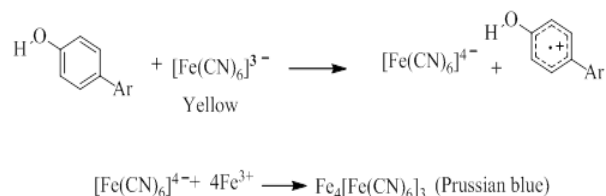
It is reported that polyunsaturated fatty acids containing 1, 4-pentadienic functional fragments are specifically sensitive to oxidation. The hydroperoxide produced from polyunsaturated fatty acids is stabilized by radical state via double-bond rearrangement (electron delocalization) which results in to conjugated dienes (Scheme 4). These compounds absorb in the ultra-violet range (235 nm and 270 nm respectively) where the absorptions are detected using spectrophotometric techniques so as to evaluate the

oxidation level [45, 46]. This way of conjugated dienes and peroxides determination have been used in a number of studies even though they do have of their own weaknesses [47-50].

Another method involves the correlation between 235 nm absorption value and peroxide value has also been reported [51]. It is the Ferric Reducing Antioxidant Power (FRAP) Assay method. It works by the reduction of Fe^{3+} to Fe^{2+} depending on the available reducing species followed by the change in color from yellow to blue. In this assay, antioxidants force the reduction of the Fe^{3+} to Fe^{2+} to occur [52, 53]. Most probably the reactions occurring during the processes are shown in Scheme 5.

These days, plant extracts are becoming the chosen antimicrobials due to the increment in resistance of the microbes and health concerns related to the use of synthetic antimicrobials [54, 55]. The antimicrobial agents have a unique mode of action which depends on microorganism cell structure like membrane structures, gram negative and gram positive bacteria [56]. Antimicrobial agents affect the microbes by interfering with cell wall synthesis, inhibiting the protein synthesis, inhibiting nucleic acid synthesis and blocking metabolic pathways so as to eliminate them [57]. The phytochemicals responsible for the antibacterial activity of a plant extract are phenolic acids and simple phenols, quinones, flavanoids, tannins and alkaloids [58-62].

An antimicrobial susceptibility test is done through methods like dilution, diffusion and bioautographic methods. These days, it is not possible to find a standard reproducible method of determining the antimicrobial activity of plant extracts and phytochemicals [63].

**Scheme 5** Reactions occurring during FRAP assay.

Diffusion method is advantageous over the quantitative methods used to determine minimum inhibition concentration (MIC). It needs less work force and smaller amounts of test agents [64]. There have been many reports involving agar-based testing of Disc-Diffusion Method as it is simple to use [65]. There are also many reports which have used the Agar Well Diffusion technique to assess the antibacterial activity of plants extract [66]. This work mainly deals with the assessment of the antioxidant and antibacterial activities of the *Aloe elegans* leaf extract which has not been reported yet.

2. Materials and Methods

2.1 Plant Material

The leaves of *Aloe elegans* plant were collected from Meshenti which is 16 Km from Bahir Dar, Amhara regional state Ethiopia in February, 2017. The plant was identified as such by Dr. Ali Seid from Bahir Dar University, department of Biology.

2.2 General

De-ionized and distilled water were used in all of the experimental works. Solvents and reagents were used as received from suppliers. Ethanol (99.5%), Methanol (99.9%) was procured from UNI-CHEM Chemical Reagents. chloroform (99%) was purchased from Fisher Scientific UK Limited, UK. Ferric chloride (99%), conc. sulphuric acid (98%), hydrochloric acid (35.4%) was obtained from Loba Chemie Pvt. Ltd, India. KI (99 %), iodine solution (80%), Benedict's solution were all purchased from Abron Chemicals, India. $K_3[Fe(CN)_6]$ (99%), Vitamin C (L-ascorbic acid) (98%), phosphate buffers (pH 6.6) and trichloroacetic acid (99%) (Merck, Darmstadt, Germany), Ammonia solution (25%), Petroleum ether, acetic acid (90%) and starch indicator were obtained from Blulux Laboratories(P) Ltd. Sunflower oil was used as an oxidizable substrate. $Na_2S_2O_3$ (99%) was purchased from Thomas Baker (chemicals), India. Muller Hilton agar was purchased from Oxoid CM, UK.

Gentamicin was purchased from Abcek adtek(P) Ltd. Ultraviolet-Visible (UV-Vis) spectrophotometer (Aglient Technologies (Cary 60 UV-Vis)) and electrical shaker (Heidolph promax 2020) were used.

2.3 Methods

2.3.1 Preparation of plant material and gel

The leaves of *Aloe elegans* plant were collected from Bahir Dar (Meshenti) in February, 2017 (Figure 2).

2.3.2 Preparation of the Extracts

Fresh *Aloe elegans* gel was collected by cutting the leaves transversally near the base and inclining on stainless tray. The gel was then dried in an oven at 80 °C for 48 h and then powdered. Ten grams of this powder was soaked separately in 100 mL of each of ethanol and methanol and shaken for 24 h. The content of each flask was then filtered using (Whitman filter paper Number 1). The solvent from each flask was then removed using a rotary evaporator. This dried extract was further powdered and then dissolved in distilled water for further analysis [67].

2.3.3 Phytochemical screening

A. Test for phenols (ferric chloride test)

Extracts were treated with 3 - 4 drops of ferric chloride solution. Bluish black color was observed showing the presence of phenols.

B. Test for Flavonoids (ferric chloride test)

0.2 mL of each extract was added to 2 mL 10% $FeCl_3$ solution and the mixture was shaken. A wooly brownish precipitate was formed showing the presence flavonoids.

C. Test for Terpenoid

Into 2 mL of aqueous solution of each extract were added few drops of chloroform followed by drop wise addition of concentrated sulfuric acid. A reddish brown

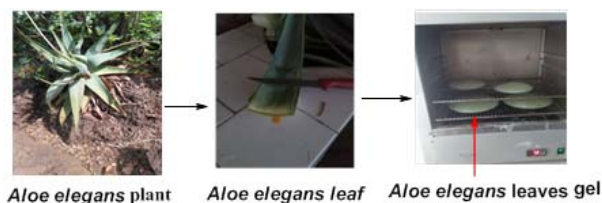


Fig. 2 Preparation of *Aloe Elegans* leave gel.

color was observed at the interface indicating the presence of terpenoids.

D. Test for Anthraquinones

2 mL of aqueous solution of each extract was taken in 10 mL of ethanol and was steamed for 5 min and filtered. 2.0 mL of the filtrate was added to 2 mL chloroform and shaken thoroughly. To the chloroform layer that was taken off, distilled water (5.0 mL) and dilute ammonia solution (5 mL) were added. The contents were then shaken. A red coloration appeared in the upper ammonia phase showing the presence of anthraquinones.

E. Test for Tannins (Ferric chloride test)

1.0 mL of each extract was stirred with 1.0 mL ferric chloride solution. A greenish black precipitate was formed showing the presence of tannins.

F. Test for Saponin

On shaking 0.2 mL of each extract taken in 5.0 mL distilled water and shaken for 20 min, foams appeared indicating the presence of saponins.

G. Test for Carbohydrate (Benedict test and Iodine test)

Treatment of each extract with few drops of Benedict's solution did not yield a brick red color expected for glucose test. Similarly, treatment of each extract with few drops of iodine solution did not yield a dark blue color expected for starch test.

H. Test for Alkaloids (Wagner's test: Iodine-Potassium iodide solution)

A solution of 1.2 g of iodine and 2 g of H_2SO_4 was diluted to 100 mL. 10 mL of alcoholic extract was acidified by adding 1.5 % v/v of HCl and a few drops of Wagner's reagent. A yellow or brown precipitate expected for alkaloids was not observed.

2.4 Determination of Antioxidant Activities

2.4.1 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay is characterized by the reduction of Fe^{3+} to Fe^{2+} . Depending on the available reducing species, alteration of color changes from yellow to blue occurs which is analyzed through a spectrophotometer

[52]. Antioxidant capacity as per reducing power assay was measured according to a method reported by Oyaizu [66].

In a typical experiment, variable concentration of extracts 2.5 mL (20 $\mu\text{g/mL}$ – 80 $\mu\text{g/mL}$) was mixed with sodium phosphate buffer (2.5 mL, 0.2 M, pH = 6.6) and 2.5 mL, potassium ferricyanide (1% w/v in distilled water). The contents were shaken to ensure proper mixing and then incubated at 50 °C in a water bath for 20 min. Following addition of trichloroacetic acid (2.5 mL, 10% w/v in distilled water), the mixture was centrifuged for 10 min. The supernatant (5 mL) was decanted and diluted with 5 mL distilled water and 1 mL ferric chloride (0.1% w/v in distilled water) solution was added and was mixed well. Blank for each solvent was run using the same procedure but replacing the plant extract with an equal volume of solvent. Absorbance was measured at 700 nm. Vitamin C (L-ascorbic acid) was used as a reference antioxidant [67].

2.4.2 Peroxide Value Determination

Peroxide values were measured by AOCS (American Oil Chemist's Society) cd 8-53 official method (1990). In a typical experiment, 10 mL of sunflower oil with or without methanol or ethanol *Aloe elegans* leaves extract was added to a 250 mL flask containing 50 mL of a mixture of glacial acetic acid and chloroform in the ratio 3:2. The solution was swirled until the sample is dissolved. After addition of freshly prepared 0.5 mL KI (10%) solution to the mixture, the contents were shaken for 1 min. Distilled water (30 mL) was then added. The mixture was then titrated with Sodium thiosulphate (0.1 N $\text{Na}_2\text{S}_2\text{O}_3$) solution using 0.5 mL of 1 % starch indicator until a color change was noted. A blank (without antioxidant) was prepared alongside oil samples. The antioxidant efficacy of the extracts was compared with Vitamin E (α -tocopherol) as a reference.

2.4.3 Conjugated Diene Assay

Into a dry 25 mL volumetric flask, 0.25 g of sunflower oil (control) was added. Each of two similar

flasks was charged with 0.248 g of sunflower oil and 0.002 g of either ethanolic or methanolic extract of *Aloe elegans* leaves gel. Into a fourth volumetric flask of same size were added 0.248 g of sunflower oil and 0.002 g vitamin E (α -tocopherol). Petroleum ether was then added up to the mark of each flask. Each flask was then swirled gently for homogenization and further diluted to 0.1g per 25 mL by addition of 37.5 mL of petroleum ether. The absorbance at 232 nm was measured for conjugated diene determination. The antioxidant efficacy of the extracts was compared with vitamin E (α -tocopherol) used as a reference.

2.4.4 Antibacterial Activity Screening Assay

The antibacterial activity of *Aloe elegans* leaves gel extract was tested using Agar Well Diffusion Technique as described by Agarry *et al* [68]. Wells approximately 5 mm diameter were cut on sterile nutrient agar plates and swabbed with an overnight broth culture of the organism.

About 50 mg/mL of the *Aloe elegans* leaves gel (methanol and ethanol extracts) were filled into each of the wells and incubated at $37\text{ }^{\circ}\text{C} \pm 0.2\text{ }^{\circ}\text{C}$. The results were compared with the antibiotic gentamicin used as a reference. Antibacterial activity in terms of zones of inhibition (mm) was recorded after 24 h. of incubation. The antagonistic action of extracts of *Aloe elegans* leaves gel and Gentamicin was tested against test organisms (*S. aureus* and *E. coli*) in triplicates [69].

2.5 Data Analysis

A. Analytical Methods

The percentage reduction power of methanol and ethanol extracts of *Aloe elegans* leaves gel were calculated using the equation given below [70].

Percentage (%) reduction power =

$$\frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{sample}}} \times 100\% \quad (\text{eq1})$$

Where A_{sample} = absorbance of sample

A_{blank} = absorbance of blank or normal complex

(OR) Percentage inhibition of the extracts and

ascorbic acid can be calculated by:

$$\text{Antioxidant Capacity (\%)} = \frac{A_0 - A_1}{A_0} \times 100\%$$

Where, A_0 is the absorbance of sample extract and A_1 is the absorbance of control or normal complex.

Peroxide values (PVs) (mmol/L) were calculated using the following formula.

$$\text{PV(mmol/L)} = \frac{V \times N \times (1000)}{\text{weight of sample}} \quad (\text{eq2})$$

Where V = consumption of 0.1N sodium thiosulfate solution in the test

N = the normality of sodium thiosulfate solution

The molar absorptivities or molar extinction coefficient at 232nm for a concentration of 1g per 100ml in a 10mm cell is calculated. The conjugate dienes contents of oil is determined by

$$\text{The } E_{1\text{cm}}^{1\%} \text{ of the oil is } E_{1\text{cm}}^{1\%} = \frac{A \times V}{\% \text{ oil in solution} \times W \times 100} \quad (\text{eq3})$$

$E = \epsilon$ = is the extinction value at a concentration of 1% in a cell 1cm, A= absorbance in a 1cm (10 mm) cell, V = the volume of the solution in ml, W= weight of the sample in gram

B. Statistical Methods

All the data for antioxidant and antibacterial activity were recorded in triplicate data as mean $\pm$ standard deviation.

3. Results and Discussion

3.1 Yield of the Extraction

The yield of methanolic and ethanolic extracts of *Aloe elegans* leaves gel obtained in the present work is 4.34% and 5.83%, respectively.

3.2 Qualitative Phytochemical Screening

The phytochemical analysis carried out on methanol and ethanol extracts of powdered *Aloe elegans* leaves gel is summarized in Table 1. As per these tests, alkaloids and carbohydrates were not detected in both the methanol and ethanol extracts of powdered *Aloe*

elegans leaves gel. Tannins and terpenoids were judged to be present to an apparently similar extent in both the extracts. The methanol extract apparently contained higher amounts of flavonoids and phenols relative to the ethanol extract. On the other hand, the saponin content in the ethanol extract was seen to be greater relative to the methanol extract (Figure 3). It is documented that the presence or absence of phytochemicals in a given plant material is greatly affected by environmental factors and degree of maturity [71].

3.3 Peroxide Value Determination

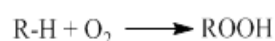
This method relies on iodometric titration to determine oxidation of fatty foodstuffs. In the initial stage of lipid oxidation, conjugated double bonds are rapidly formed due to abstraction of hydrogen from allylic sites in poly unsaturated fatty acid (PUFA) [38]. In this study, efficacy of ethanol and methanol extracts of *Aloe elegans* leaves gel to prevent or delay the onset of oxidation of sunflower oil is measured using procedures reported in the literature [39].

The peroxide value is determined by measuring the iodine liberated from potassium iodide by peroxide, using sodium thiosulfate solution as the titrant. In the presence of acetic acid, the reaction scheme for hydroperoxides in sunflower oil is shown in Scheme 10.

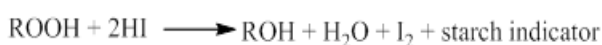
The reaction resulted in color change from bright yellow to colorless upon addition of sodium thiosulfate (Figure 4).

Table 2 shows the variation in peroxide value at room temperature of sunflower oil with or without added antioxidants. The change in peroxide value was

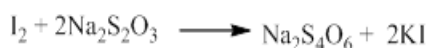
Generation of hydroperoxide



Generation of iodine



Titration step



Scheme 10 Reaction equations for hydroperoxide determination.

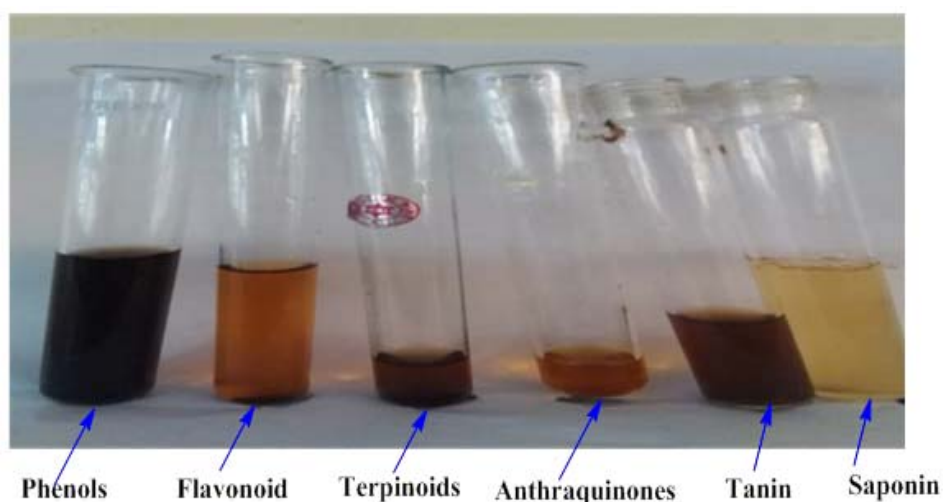


Fig. 3 Qualitative Phytochemical screening Methanolic and Ethanolic extracts of *Aloe elegans* leaves gel.

Table 1 Qualitative photochemical analysis of methanolic and ethanolic extracts of *Aloe elegans* leaves gel.

Solvent used	Alkaloid	Flavonoids	Phenols	Saponnins	Tannins	Terpenoid	Carbohydrates
Methanol	-	++	++	+	+	+	-
Ethanol	-	+	+	++	+	+	-

++ = higher intense and + = lower intense

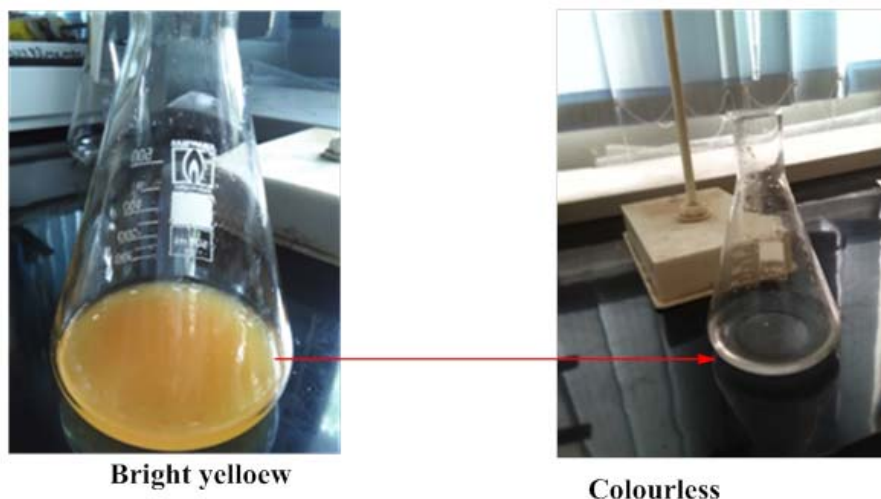


Fig. 4 Sunflower (SFO) color change.

Table 2 Relative decrease in peroxide value of SFO by the addition of antioxidants.

ST	Control SFO	Vitamin E + SFO	Ethanol extract + SFO	Methanol extract + SFO
1	15±0.542	13±0.064	10±0.023	8±0.0178
3	25±0.125	23±0.076	20±0.0560	16±0.0348
6	35±0.437	32±0.0471	28±0.0533	26±0.0870
12	45±0.676	43±0.0564	38±0.0298	36±0.128

All data are expressed as mean (n=3) ± SD (standard deviation)

monitored over a period of 12 days. It is seen that the highest peroxide value (45 ± 0.67 mmol/L) was observed for sunflower oil containing no added antioxidant (C-SFO) while lower peroxide values were observed for SFO containing extracts of *Aloe elegans* leaves gel and vitamin E. The lower peroxide value observed for sunflower oil containing *Aloe elegans* leaves gel extracts relative to the control indicate the ability of the extracts in preventing the oxidation of oils. In other words, the results indicate higher rate of oxidation of sunflower oil containing no added antioxidants. It is also noted in this work that the peroxide values of sunflower oil containing methanolic extract of *Aloe elegans* leaves gel is less than that in the sample containing ethanolic extract suggesting better ability of the methanolic extract in preventing oxidation of the oil (Figure 5). The results clearly show that both methanolic and ethanolic extracts of *Aloe elegans* leaves gel delay the onset of aerial oxidation of sunflower oil relative the oil having no added antioxidants albeit the former showing a better ability

to prevent oxidation of the oil. It is interesting to note that, at the same concentration, both the methanolic and ethanolic extracts of *Aloe elegans*

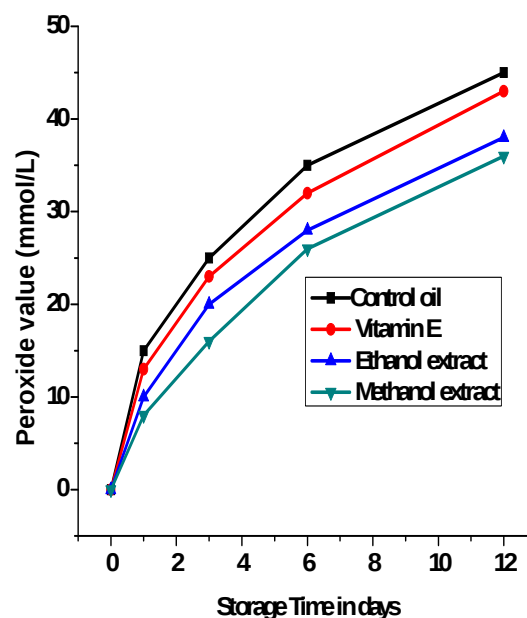


Fig. 5 Effect of Methanolic, Ethanolic *Aloe elegans* leaves gel extract and vitamin E on peroxide formation in comparison with control SFO at room temperature.

leaves gel exhibited better ability than the familiar antioxidant vitamin E in preventing oxidation of sunflower oil. This suggests that *Aloe elegans* leaves gel is rich sources of antioxidants.

3.4 Antioxidant Activity by Reducing Power Assay

Reducing power (or, antioxidant capacity) of the methanolic and ethanolic extract of *Aloe elegans* leaves gel and ascorbic acid were determined. As shown in (Figure 6), and in line with the results observed in the peroxide assay, the methanolic extract displayed higher reducing power than ethanolic extract, but both extracts shown higher potency relative to the standard ascorbic acid. All extracts and the standard had shown almost

similar increasing trend in reducing power with the increase in the concentration (Figure 7).

In this assay, the presence of reducers (*i.e.*, antioxidants) causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the Fe^{2+} concentration [72].

As can be seen from Table 3 and Figure 7, the absorbance as well as reducing power of methanolic and ethanolic extracts of *Aloe elegans* leaves gel and the standard ascorbic acid increases as the concentration increases from 20 $\mu\text{g/mL}$ -80 $\mu\text{g/mL}$. The percentage reduction power of the methanolic and ethanolic *Aloe elegans* leaves gel extract and the

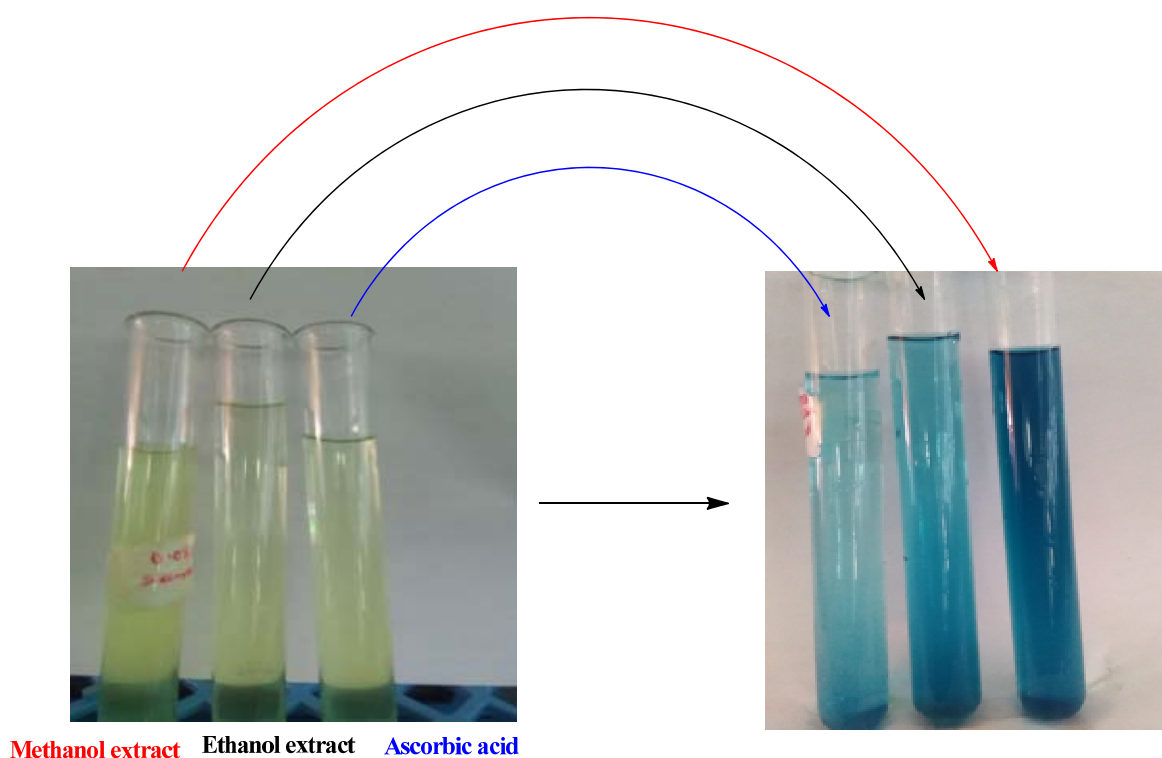


Fig. 6 Formation of Perl's Prussian blue after the addition of FeCl_3 .

Table 3 Absorbance and percentage reducing power of extracts and the standard (abs at 700 nm).

Conc. of extract in $\mu\text{g/mL}$	Abs of Ascorbic acid	Abs of Ethanol extract	Abs of methanol extract	% RP of Ascorbic acid	% RP of Ethanol extract	%RP of Methanol extract
20	0.234 $\pm$ 0.0002	0.256 $\pm$ 0.0054	0.324 $\pm$ 0.0015	59.40 $\pm$ 002	62.892 $\pm$ 0.00054	65.32 $\pm$ 0015
40	0.368 $\pm$ 0.0015	0.383 $\pm$ 0.0052	0.421 $\pm$ 0.0002	74.18 $\pm$ 0015	75.190 $\pm$ 00052	77.431 $\pm$ 0002
60	0.435 $\pm$ 0.0013	0.485 $\pm$ 0.0017	0.524 $\pm$ 0.0013	78.16 $\pm$ 0013	80.412 $\pm$ 0017	81.870 $\pm$ 0013
80	0.4866 $\pm$ 0.0021	0.523 $\pm$ 0.0021	0.595 $\pm$ 0.0001	80.47 $\pm$ 0021	81.830 $\pm$ 0001	84.034 $\pm$ 0001

All data are expressed as mean ($n = 3$) $\pm$ SD (standard deviation)

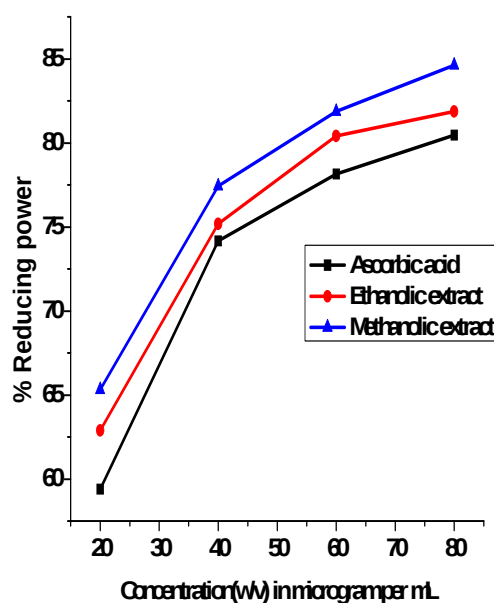


Fig. 7 Reducing power assay of extracts and ascorbic acid as a function of concentration.

ascorbic acid were calculated using (equation 2.1). It is also noted that the reducing power of the methanolic and ethanolic *Aloe elegans* leaves gel extract was relatively higher than the standard antioxidant ascorbic acid at the same concentration.

3.5 Peroxide Value Determination

Unsaturated fatty acids undergo free radical-induced oxidation to afford conjugated dienes (CD) [73]. Spectrophotometric measurement of molar absorptivity or molar extinction coefficient ($M^{-1}cm^{-1}$ or $g^{-1}cm^{-1}mL$) at 232 nm is used to monitor CD formation. The absorption at the wavelength specified is due to the presence of conjugated diene systems which result from lipid peroxidation and subsequent rearrangement of double bonds.

Table 4 summarizes the molar absorptivity or molar extinction coefficient ($M^{-1}cm^{-1}$ or $g^{-1}cm^{-1}mL$) to monitor conjugated dienes in sunflower oil with or without added antioxidants over a period of one day at room temperature. It is seen that the conjugated dienes in sunflower oil containing methanolic and ethanolic extracts *Aloe elegans* leaves gel and vitamin E is less than that in the control SFO, showing the antioxidant

efficacy of the extracts and vitamin E. It is seen here too that both methanolic and ethanolic extracts of *Aloe elegans* leaves gel show greater antioxidant efficacy relative to vitamin E, the methanolic extract showing greater antioxidant potency.

3.6 Antibacterial Activity Determination

In this study the antibacterial activity of methanol and ethanol *Aloe elegans* leaves gel extracts was investigated using agar well diffusion technique against two selected human pathogens, *E. coli* and *S. aureus*.

For the antibacterial tests by Muller Hinton agar (MHA) containing bacterial strain, approximately 5 mm diameter wells were made on the surface of the MHA plate and wells were filled with approximately 50 mg/mL of antibiotic agent (extracts) and then the plate was incubated for 24 h at 37 °C. After 24 hours incubation clear zone around the plate was observed in the methanolic and ethanolic extract, which is an indication for its antibacterial activities.

Results of antibacterial activity of all the extracts and their efficacies as compared to gentamicin used as a standard are shown in Table 5 and Figure 8. As can be observed from the table both methanol and ethanol *Aloe elegans* leaves gel extracts exhibit antibacterial activity against the selected gram positive and gram negative bacteria.

The ethanol extract showed better ability to inhibit *S. aureus* relative to the methanol extract with inhibition zone of 14 mm and 12 mm, respectively (Figure 9). On the other hand, the methanol extract of *Aloe elegans* leaves gel showed better bactericidal effect on *E. coli* relative to that of the ethanol extract, with inhibition zones of 10 mm and 6 mm, respectively. However the antibacterial activity of both methanol and ethanol extract is less than the standard antibiotics gentamicin in both types of bacterial strain. The standard antibiotic gentamicin (10 μg /disk) shows the highest inhibition zone in both *S. aureus* and *E. coli* bacterial strain (21 mm and 19mm respectively).

Table 4 Molar absorptivity or molar extinction coefficient ($M^{-1}cm^{-1}$ or $g^{-1}cm^{-1}mL$) at 232 nm of SFO over a period of one day at room temperature with and without antioxidants.

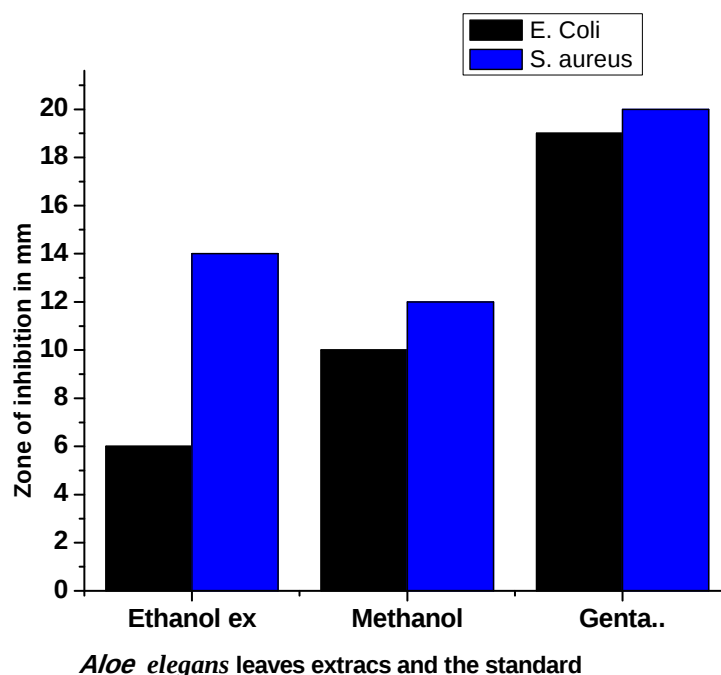
Days	CD SFO	CD Vitamin E + SFO	CD ethanol extract + SFO	CD methanol extract + SFO
1	4.63±0.0032	4.306±0.003	3.387±0.0021	3.006±0.0045

All data are expressed as mean (n = 3) ± SD (standard deviation)

Table 5 Antibacterial activity of *Aloe elegans* gel extracts against selected pathogenic bacteria.

Organism	Mean zone of inhibition in mm		
	Methanol extract	Ethanol extract	Gentamicin standard antibiotic
Gram negative (<i>E. coli</i>)	10±0.0342	6±0.0215	19±0.0417
Gram positive (<i>S. aureus</i>)	12±0.0531	14±0.0317	21±0.0218

All data are expressed as mean (n = 3) ± SD (standard deviation)

**Fig. 8** The antibacterial activities of *Aloe elegans* leave extracts and standard Gentamicin antibiotic on *E. coli* and *S. aureus*.

The result of antibacterial activity of both methanolic and ethanolic extracts of *Aloe elegans* leaves gel could be compared with the antibacterial activities of the methanolic and ethanolic extracts of other parts of this plant reported by Rubina Lawrence *et al.* [71]. Using methanol extract a zone of inhibition of 13 mm in *E.coli* and 14 mm in *S. aureus* and in ethanol extracts a zone of inhibition of 12 mm in *E.coli* and 15 mm in *S.aureus* has been observed [69].

The difference in antibacterial activity for methanolic and ethanolic extracts of *Aloe elegans* leaves gel is most probably due to difference in phytochemical content between the solvents. For

example pyrocatechol methanol extract was illustrated by Cowan. Pyrocatechol is a hydroxylated phenol, known to be toxic to micro-organisms. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to micro-organisms and the increase in hydroxylation which further results in increase in toxicity. Further, phenolics also act by denaturing proteins and disrupting cell membranes. And *p*- coumaric acid from ethanol extract illustrated by Rubina Lawrence *et al.* increase the lag phase of the micro-organisms and is also able to inhibit the enzymatic activity of the micro-organisms [74].

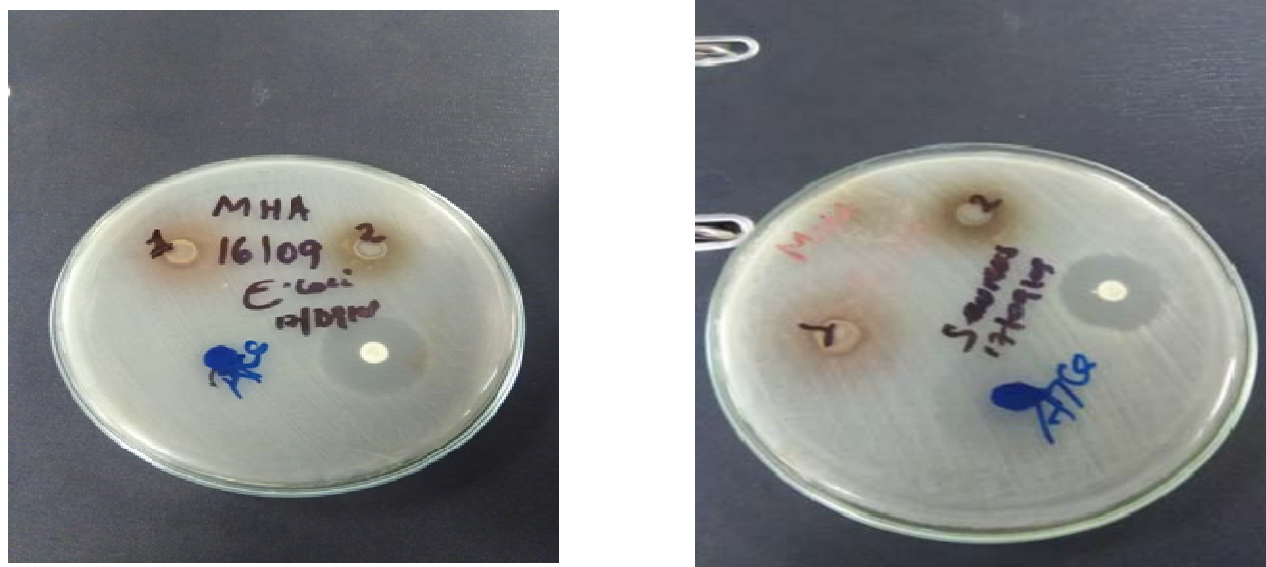


Fig. 9 Growth inhibition of *E.coli* and *S.aureus* by methanolic extract indicated by number 1, ethanolic extract indicated by number 2 and the standard antibiotic Gentamicin using agar well diffusion technique.

4. Conclusions and Recommendation

In all the antioxidant assays studied in the present work, both the methanolic and ethanolic extracts of *Aloe elegans* leaves gel exhibited antioxidant activities. In the FRAP assay, both the methanolic, ethanolic extracts of *Aloe elegans* leaves gel were able to reduce $K_3[Fe[(CN)_6]]$ to $K_4[Fe[(CN)_6]]$. Likewise, both extracts were found to protect sunflower oil from aerial oxidation. It is noteworthy that *Aloe elegans* leaves gel extract were found to inhibit sunflower oxidation better than Vitamin E. This has been inferred from the observation that the absorption coefficient for conjugated dienes formed from Sunflower oil (SFO) was less in magnitude in the presence of the *Aloe vera* leaves gel extract than in the presence of Vitamin E. The highest peroxide value was also observed for control sunflower oil sample while lower peroxide values were observed for SFO containing *Aloe elegans* leaves gel extract and vitamin E. Lower peroxide value for SFO with *Aloe elegans* leaves gel extract indicated effectiveness of the extract in preventing oxidation of oils. The antibacterial potential of the extracts was determined against *Escherichia coli* and *Staphylococcus aureus*. Towards all

the microbes, the standard Gentamicin was the most potent. *Staphylococcus aureus* were the most susceptible strain, with almost all antibiotics.

This study clearly shows that *Aloe elegans* leaves gel have significant antioxidant and antibacterial activities – a property that may underlie the widespread use of the plant in folk medicine. These properties might be due to the phytochemical constitution of the leaves. Hence our final opinion is that this paper will have of its own contribution on the study of utilizing *Aloe elegans* plant leaves for medical applications.

Data Availability

Every data used to support the findings of the study are available from the corresponding author upon the request.

Conflict of Interest

The authors declare no conflict of interest.

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